Food Hydrocolloids xxx (2018) 1–10

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Effects of folic acid esterification on the hierarchical structure of amylopectin corn starch

Pallab Kumar Borah [a, b], Michael Rappolt [a], Raj Kumar Duary [b], Anwesha Sarkar [a, *]

[a] Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, LS2 9JT, United Kingdom
[b] Department of Food Engineering and Technology, School of Engineering, Tezpur University, Napaam, Assam, 784 028, India

ARTICLE INFO

Article history:
Received 27 September 2017
Received in revised form
5 February 2018
Accepted 13 March 2018
Available online xxx

Keywords:
Starch
Folic acid
Multiscale structural analysis
Self-assembly
SAXS

ABSTRACT

There are burgeoning research interests in designing biocompatible colloidal delivery systems for treating as well as delaying the recurrence of chronic diseases, including various forms of cancers. In this respect, folic acid (FA) esters and starch are particularly interesting owing to (i) the molecular recognition of FA by folate receptors and (ii) the biocompatibility of starch based delivery systems. In this study, the effects of esterification of amylopectin corn starch (ACS) with FA using an n, n'-dicyclohexylcarbodiimide/4-dimethylaminopyridine (DCC/DMAP) mediated esterification reaction were investigated at multiple length scales. Scattering (light, X-ray), spectroscopy (FTIR), electrophoretic mobility (ζ-potential) and confocal laser scanning microscopy (CLSM) confirmed that structural rearrangements (short- and long-range) occurred in the starch-folic acid ester (SF) derivatives with increased FA content (degree of substitution, 0.01–0.05). The SF ranged in size from 200 to 600 nm and were negatively charged (ca. −24 mV, SF20). FTIR revealed a loss of double-helical structure on FA substitution. Notably, CLSM and small angle X-ray scattering (SAXS) both showing an FA-assisted self-assembly and crosslinking of SF, later confirming columnar assemblies with unit cell parameter of 4.5 nm. The wide-angle X-ray scattering (WAXS) and X-ray diffraction (XRD) pattern ($2\theta = 6.1°$, 7.7°, 13°, 17°, 20°, 22°, and 25°) in SF further gave evidence for the formation of hybrid B and V-type polymorphs, where SF may accommodate FA within a larger hybrid hexagonal lattice. This study provides structural insights for developing tunable starch-folic acid derivatives for potential applications as delivery vehicles for pharmaceuticals and nutraceuticals targeting folate receptors.

1. Introduction

There is a continuing scientific and industrial interest in designing biocompatible colloidal delivery systems for delaying the onset as well as treatment of chronic diseases, including various forms of cancers. Starch, which is the second most abundant hydrocolloid, has been recently explored for the preparation of relatively inexpensive biocompatible delivery vehicles applying physical and chemical treatments (Ahmad, Akhter, Anwar, & Ahmad, 2012; Kim, Seo, & Lim, 2013; Li, Shin, Lee, Chen, & Park, 2016; Shalviri et al., 2012). These nano- or sub-micron-sized modified starch-based delivery systems are promising for nutraceutical and pharmaceutical applications owing to their large surface area-to-volume ratio, but generally suffer from lack of cellular specificity and molecular recognition. The molecular recognition of these nanoparticles can be greatly improved by attachment of high-affinity targeting ligand molecules. Folic acid (an oxidized form of folate), a naturally water-soluble vitamin, is such a widely explored targeting ligand molecule. Due to its high binding affinity ($K_d \sim 10^{-10}$ M) along with its specific binding properties to folate receptors in the human cells, it improves the targeting properties to the cancer cells of breast, lung, kidney, colon and brain, that are known to overexpress folate receptors by 100–300 times as compared to that of non-cancerous cells (Antony, 1996; Kamen & Capdevila, 1986). Thus, there has been significant research efforts to esterify folic acid to modify starch via a wide variety of chemical synthesis routes.

Folate esterified to polyethylene glycol (PEG) using n, n'-dicyclohexylcarbodiimide (DCC) and n-hydroxysuccinimide (NHS)-mediated esterification was conjugated to the surface of modified starch nanoparticles, latter designed via a water-in-oil

* Corresponding author. Food Colloids and Processing Group, School of Food Science and Nutrition, University of Leeds, Leeds, LS2 9JT, UK.
E-mail address: A.Sarkar@leeds.ac.uk (A. Sarkar).

https://doi.org/10.1016/j.foodhyd.2018.03.028
0268-005X/

microemulsion templating by Xiao et al. (2006). An increase in particle size of the starch nanoparticles was specifically observed upon folic acid esterification (from 50 to ~130 nm) with a folic acid content of 0.8 μg/mg of PEG-Starch nanoparticle. In another instance, folic acid was esterified to hydrophobized pullulan, an exopolysaccharide derived from starch, using DCC and 4-dimethylaminopyridine (DMAP) mediated chemistry to produce nanoparticles (Zhang et al., 2010). Folic acid esterification resulted in increasing the hydrodynamic diameter of the pullulan acetate nanoparticles from ca. 185 nm to 261 nm. Such increase was attributed to the enhanced swelling of the folate-pullulan esters in aqueous dispersion, which was driven by the hydrophilic nature of the folic acid. Folic acid conjugated to hydroxyethyl starch nanocapsules via n-(3-dimethylaminopropyl)-n′-ethylcarbodiimide hydrochloride (EDC)-mediated esterification (Baier et al., 2012) also showed a similar behaviour of increasing the particle size of starch from 275 nm to 307 nm.

On the other hand, no significant changes in the hydrodynamic diameter of aminated starch was observed by Saikia, Das, Ramteke, and Maji (2017), when folic acid was esterified to aminated starch/ZnO coated iron oxide nanoparticles using an NHS/EDC mediated esterification reaction. It appears from the aforementioned studies that esterification of folic acid resulted in modification of starch nanoparticles at colloidal scale; however, rare attention has been given in literature to understand mechanistically, if such esterification has resulted in any structural rearrangements in starches.

Starch consists of two polymeric units, namely linear amylose composed entirely of D-glucose units joined by α-1,4-glycosidic linkages, and, extensively branched amylopectin composed of glucose units linked primarily by α-1,4-glycosidic bonds with occasional α-1,6-linkages forming the branching points (Zobel, 1988). The amylopectin and amylose polymers (glucose extension ~0.1 nm) are arranged as alternating lamellae (~10 nm) of rigid mesogen units (liquid crystalline) and flexible spacer units (amorphous). The crystalline regions consist of double helices of amylopectin in ordered arrays. Additionally, single, left-handed helix are also observed. These types may (Tan, Flanagan, Halley, Whittaker, & Gidley, 2007) or may not (Borah, Deka, & Duary, 2017) include copolymers within the helical channel.

On the other hand, folic acid although hydrophilic in nature, has a tendency to self-assemble into tetramer structures even at concentrations as low as 0.1% (w/w) via hydrogen bonding and stacking interactions, which further arrange into ordered mesophases (Bonazzi, DeMorais, Gottarelli, Mariani, & Spada, 1993; Ciuchi et al., 1994). Additionally, Kamikawa, Nishii, and Kato (2004) reported the formation of non-symmetric supramolecular assemblies in folic acid derivatives, which were synthesized using EDC/DMAP mediated esterification. The self-assembled columns of the folic acid derivatives were thought to be formed via the secondary cooperative interactions, involving hydrogen bonding, ion dipolar interactions, stacking interactions, and segregation into nanophases of molecular block structures. Hence, it is plausible that during esterification with folic acid, starch may undergo a folic acid assisted structural reorientation.

Since such structural rearrangements might result in changes of the properties of the delivery system and its release kinetics, it is vital to gain fundamental understanding of the multiscale structure of starch on esterification with folic acid, which has not been reported in literature until now. Such crucial insights will enable the optimisation of future design and fabrication of folic-acid-functionalized, colloidal starch delivery vehicles tailored for targeted drug and nutraceutical delivery applications.

In this study, we have designed different starch-folic acid esters focusing mainly on the structural rearrangements of the amylopectin corn starches mediated by esterification with folic acid. Amylopectin corn starch was utilized as the starch model, since it is devoid of amylose and thus was expected to provide distinct peaks for the lamellar phases in X-ray scattering studies. We hypothesize that controlling the degree of folic acid esterification will profoundly alter the hierarchical structure of starch particles, including colloidal properties (size, charge) and its molecular properties (lamellar structure and crystalline structure). A combination of complementary techniques of dynamic light scattering (DLS), small-angle and wide-angle X-ray scattering (SWAXS), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, electrophoretic mobility and confocal laser scanning microscopy (CLSM) were assessed to understand the effect of folic acid esterification on the structure of starch. To the best of our knowledge, this is the first study that systematically characterizes the structural rearrangements of starch on multiple length scale. This is the first in a series of papers by the present authors on the structure-function relationship of folic acid-starch esters and its overall implications towards designing biocompatible colloidal vehicles for delivery of pharmaceuticals and nutraceuticals targeted at cancer cells.

2. Materials and methods

2.1. Materials

Amylopectin corn starch (ACS) was obtained from Sigma-Aldrich Company Ltd., Dorset, UK. The ACS contained no amylose as assessed using colorimetric procedure (Morrison & Laignelet, 1983), which was in agreement with the supplier's specification. Folic acid (FA), n, n'-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich Company Ltd., Dorset, UK. Milli-Q water purified using a Milli-Q apparatus (Millipore Corp., Bedford, MA, USA) was used throughout the experiments. All other chemicals were of analytical grade unless otherwise stated.

2.2. Preparation of starch-folic acid esters

Starch folic-acid (SF) ester derivatives were synthesized using an esterification reaction between the carboxyl group of folic acid (FA) and the hydroxyl group of starch (ACS) as described previously for synthesis of stearate-grafted dextran (Du, Weng, Yuan, & Hu, 2010), with some modification. The “zero length” crosslinker, n, n'-dicyclohexylcarbodiimide (DCC) served as the coupling reagent, and, 4-dimethylaminopyridine (DMAP) was the reaction catalyzer. The SF esters with the different degree of substitutions of FA were synthesized by controlling the feed ratios of FA to starch.

Briefly, 1g FA was dissolved in 30 mL anhydrous DMSO, and, DCC, DMAP were added in the FA:DCC:DMAP molar ratio of 1:1:0.3. Activation of the FA carboxylic groups was achieved by stirring the solution for 30 min at 30 °C while maintaining dark conditions. Following this, starch was added in various concentrations to the FA solution (5–30 wt% of FA to starch dry weight) and was reacted in the dark at 30 °C for the next 24 h. The DMAP was removed by washing the reaction products first with 1N HCl and then with Milli-Q water using a Whatman No. 4 filter paper. The exposure time to 1N HCl was <5 min to avoid any degradation of the starch polymer. The reaction product was then dialyzed (3.5 kDa MWCO) against 10 mM phosphate buffer at pH 7.4 containing 0.10 M NaCl for 24 h, and, then with water for another 24 h to remove any unbounded FA and DCC. The samples were then lyophilized for 48 h, ground to a fine powder using mortar and pestle, and the SF ester derivatives (SF5, SF10, SF20, and SF30) were obtained. Control samples included the native amylopectin corn starch, ACS; ACS treated with DMSO, S/DMSO; ACS reacted with DCC and DMAP in DMSO but without FA substitution, S/DCC.

Please cite this article in press as: Borah, P. K., et al., Effects of folic acid esterification on the hierarchical structure of amylopectin corn starch, *Food Hydrocolloids* (2018), https://doi.org/10.1016/j.foodhyd.2018.03.028