Colloids and Surfaces B: Biointerfaces xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Amylose-fatty acid inclusion complexes as examined by interfacial tension measurements

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ARTICLE INFO

Article history:
Received 2 April 2015
Received in revised form 18 June 2015
Accepted 25 June 2015
Available online xxx

Keywords:
Amylose–fatty acid complexes
Capric acid
Myristic acid
Oleic acid
Alkaline pH
Interfacial tension

ABSTRACT

Amylose forms complexes with fatty acids under certain conditions, these complexes affect the functional properties of foods and could be potentially used as delivery systems of essential fatty acids in the human organism. This work uses dynamic and equilibrium interfacial tension measurements in order to investigate these complexes. First, the interfacial tension at the water/air interface under the conditions of complex formation (KOH 0.1N, pH = 12.7) was measured for three fatty acids (capric, myristic and oleic) at different concentrations. Then, amylose–fatty acid complexes were formed at three different fatty acid to amylose ratios covering a range above and below the saturation concentration of the amylose helix. For all examined systems the dynamic interfacial tension of the mixed amylose–fatty acid solution was significantly higher than this of the fatty acid solution, showing that some of the fatty acid was no longer available to adsorb at the interface and suggesting its inclusion in the complex. Besides, the dynamic interfacial tension of the mixed system was lower compared to the pure amylose solution indicating that some of the fatty acid did not participate in the complexes even at fatty acid/amylose ratios well below the saturation concentration of amylose. Using the isotherm of the three fatty acids it was shown that the fatty acid excess depended on fatty acid-to-amylose ratio.

1. Introduction

Starch consists of two homopolysaccharides amylose, an essentially linear molecule and amylopectin a heavily branched molecule. Amylose exhibits a unique behaviour among all natural polymers which is its ability to spontaneously interact, under suitable conditions, with a variety of straight chain linear polar and non-polar molecules e.g., fatty acids, fatty alcohols, monoglycerides etc. to form helical inclusion complexes where the guest molecules are confined inside the helical cavity [1–3]. In the presence of ligands (fatty acids, fatty alcohols, monoglycerides etc.) amylose undergoes a conformational change from coil to a single, left-handed helix and guest molecules enter the central cavities of amylose helices during complex formation. The amylose helix is hydrophilic on the outside and hydrophobic inside its cavity [4]. In this form the amylose inclusion complex is referred to as V-amylose [5]. The aliphatic part of the fatty acid is included inside the amylose helix, whereas the polar group is outside, due to steric hindrance and electrostatic repulsion which prohibit the polar group to enter the helical cavity [1]. The complex formation is strongly influenced by the type of fatty acids involved, but also by the conditions under which the complexes are formed [6]. The structure and the physicochemical properties of these complexes have been extensively studied by several researchers [7–11]. The formation of the amylose-inclusion complexes has been shown to inhibit the swelling of the granules during heating, to increase the temperature of gelatinization [12], to prevent leaching of amylose from the granules and to act as antistalling agents [13]. Additionally, complex formation influences important phenomena, such as rheological behavior [14] and digestion rate [15–16]. Furthermore, it was shown [10] that amylose complexes with bioactive fatty acids (e.g. 18:2ω6 conjugated linoleic acid) stabilize fatty acids against oxidation but also act as carriers of the ligand in the intestine.

To date, various methods have been employed to investigate the complexation process using X-ray Crystallography [10,17], Differential Scanning Calorimetry (DSC) [8,9,11], Scanning Electron Microscopy (SEM) [17] and Fourier Transform Infrared Spectroscopy (FTIR) [18]. Almost 30 years ago, and despite the limits of instrumentation the works of Bulpin [19–20] working with fatty acid-amylose complex formation in DMSO solutions and Raphaelides (PhD) [21] working with complex formation in 0.01N KOH solutions showed that valuable information on these systems

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http://dx.doi.org/10.1016/j.colsurfb.2015.06.053
0927-7765/

Table 1
Purified amylose properties.

Property	Value
Apparent amylose concentration	95.0 (±0.1) % (w/w)
Viscosity average Molecular weight	2.7×10^5
Water content	8.58 (±0.3) % (w/w)
Protein	0.09(±0.01) % (w/w)

Reported values are the mean of 3 replications.

can be obtained via interfacial tension measurements. To the best of our knowledge there is no recent work investigating amylose-fatty acid complex formation although better insight can be gained with the current methods and instruments. The present work aims to investigate complexes formed between amylose and fatty acids via dynamic and equilibrium interfacial tension measurements. Complexes formed between 3 different fatty acids, namely capric ($C_{10:0}$), myristic ($C_{14:0}$) and oleic ($C_{18:1}$), and amylose extracted from pea starch were investigated at different fatty acid to amylose concentrations. Furthermore, results on dynamic and equilibrium surface tension of fatty acids on alkaline pH (KOH 0.1 N, pH = 12.7) are presented.

2. Materials and methods

2.1. Materials

The preparation of amylose-fatty acid complexes was carried out using amylose extracted from dry pea seeds. The water content, protein content, apparent amylose concentration and amylose molecular weight of amylose were determined. The water content of amylose was determined using the AACC method 44-15A [22]. Nitrogen content was determined by standard Kjeldahl methodology [23]. Apparent amylose content which reflects the purity of amylose, was determined using the method of Morrison and Laignelet [24]. The molecular weight of amylose was determined using an Ubbelohde capillary viscometer according to the method of Greenwood [25]. The properties of amylose are presented in Table 1. Capric (10:0) (purity ≥ 98%, Sigma), myristic (14:0) (purity > 99%, Sigma) and oleic (18:1) acids (purity > 99%, Fluka) were used without further purification. All solutions were prepared using filtered ultrapure water (Purelabtex, EIGA Process Water, Marlow, UK; resistivity at 25 °C: 18MΩ.cm). Titration ampoule (Dilut-it, analytical concentration, J.T.Baker) was used for the preparation of KOH solution. The absence of surface active impurities in the KOH solution was verified by surface tension measurements.

2.2. Formation of V-amylose molecular inclusion complexes

The preparation of the V-amylose complexes was based on a method proposed by Karkalas et al. [9]. Briefly, amylose was dissolved, in alkaline KOH solution and then mixed together with the fatty acid solution. That is, 600 mg of amylose was dissolved in 40 ml 0.1 N potassium hydroxide (KOH) solution. Solubilization initially performed at 25 °C under continuous stirring for 24 h, followed by increasing the temperature to 90 °C for 2–3 min. Then the solution of the amylose was cooled to 30 °C. As for the dissolution of the fatty acids, 60 mg of fatty acid was dissolved in 0.1N KOH (60 ml) under constant stirring at 90 °C until the fatty acid was dissolved completely. Then the solution of the fatty acid was cooled to 30 °C. The amylose and fatty acid solutions, were then mixed to form complexes at 30 °C. Complexes at three different fatty acid-to-amylose ratios were prepared: 10% (as described above), 1% and 0.1%. For the 1% and 0.1% fatty acid to amylose ratio the concentration of amylose in the solution was the same as for the 10% fatty acid to amylose ratio solution and the concentration of fatty acid varied accordingly. An attempt was also made to prepare systems using palmitic (C16:0) and stearic acid (C18:0). The fatty acid solutions were clear at 90 °C but soon became turbid at 25–30 °C and no measurements using these systems were performed. Regarding myristic acid (C14:0) when measurements were performed at 15–20 °C using freshly prepared solutions the measurement of surface tension as a function of time manifested non-typical behavior. The surface tension initially decreased, then formed a longer or shorter plateau (depending on temperature) and then it increased again as a function of time. Such results may be attributed to crystallization/dissolution problems. Two typical examples are given in Fig. S1.

2.3. Measurement of equilibrium and dynamic interfacial tension at the air/water interface.

Two methods were used to measure interfacial tension at the air/water interface: (a) the Wilhelmy Plate method and (b) the pendant drop method/axisymmetric drop shape analysis. For the pendant drop method a Cam 200 by KSV instrument was used and analysis was performed via the Young–Laplace equation for the fitting using One Attension software (Version 1.8, Biolin Scinetific). Measurements were conducted for a period of 800 s after which evaporation of the pendant drop became significant. Measurements using the pendant drop method where coupled by the Wilhelmy plate method (Sigma 701, One Attension) at continuous mode at an acquisition rate of 1 Hz. The time of the first recorded measurements using Sigma 701 instruments was approximately 10 s after the formation of the fresh interface whereas for the pendant drop instrument this time was limited to 1–2 s. Measurements were performed in the system after the formation of the complexes but also in pure amylose solution and pure fatty acid solutions at different concentrations. All measurements for the complexes were performed immediately after solution/complex preparation.

3. Results and discussion

Fig. 1 presents results of surface tension of decanoic, myristic and oleic acids and amylose all in a 0.1 N KOH solution (pH = 12.7). To our knowledge there is no information in the literature on the dynamic interfacial tension of fatty acids in such a high pH. Most studies referring to fatty acids use acidic pH where the non-dissociated form of fatty acids is present in the aqueous solution [26–30]. It is well known that the two forms (dissociated, non-dissociated) of fatty acids differ both in solubility and surface activity. According to the present literature different studies with different fatty acids have shown opposing trends on the effect of pH of fatty acid surface activity [27,31].

The pendant drop method is a very good method from which the dynamics of adsorption of surfactants can be studied and this for adsorption times starting from 1 to 2 s and higher. Nowadays the Wilhelmy plate instruments provide the possibility of continuous measurements and therefore the dynamics of adsorption can be studied at times starting form approximately 10 s and higher. In addition the Wilhelmy plate method is most appropriate for equilibrium measurements. For the fatty acids the higher the non-polar carbon chain length the higher the decrease induced in surface tension for the same mass concentration (similar molar concentration) (Fig. 1) which is in accord with Traube's rule [32] As expected the change in the fatty acid concentration in the solution affects both the dynamics of adsorption as well as the equilibrium value with oleic acid reaching the Critical Micelle Concentration (CMC) (Fig. 1c) at lower concentrations compared to the other two fatty acids which have not reached CMC at the same mass concentrations

Please cite this article in press as: A. Marinopoulou, et al., Amylose-fatty acid inclusion complexes as examined by interfacial tension measurements, Colloids Surf. B: Biointerfaces (2015), http://dx.doi.org/10.1016/j.colsurfb.2015.06.053