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Complexation of bovine serum albumin and sugar beet pectin: Stabilising oil-in-water emulsions

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ABSTRACT

In a previous study (Langmuir 28 (2012) 10164–10176.), we investigated the complexation of bovine serum albumin (BSA) with sugar beet pectin (SBP). A pH–composition phase diagram was established and structural transitions in relation to the phase diagram during complexation were identified. The present study examines the implications of these interactions on the emulsifying performance of BSA/SBP mixtures. Middle-chain triglycerides (MCTs) in water emulsions were prepared using conditions corresponding to different regions of the phase diagram. At high pHs and in the stable region of mixed individual soluble polymers where complexation is absent, there is no improved emulsifying performance, compared with the individual protein and polysaccharide. For these mixtures, the emulsion characteristics are controlled by the major component in the solutions, as determined by the competitive adsorption of the two components at the oil–water interface. At low pHs and low BSA/SBP ratios, and so mainly within the stable region of intramolecular soluble complexes, BSA/SBP mixtures greatly improve the stability of emulsions. Here, stabilisation is controlled by the cooperative adsorption of the two components at the oil–water interface. Through electrostatic complexation BSA promotes the adsorption of SBP on to interfaces to form a thick steric layer around emulsion droplets and thus providing better stability. At low pHs and high BSA/SBP ratios, that is, mainly within the unstable region of intermolecular insoluble complexes, emulsions prepared are extremely unstable due to bridging flocculation between emulsion droplets.

1. Introduction

Protein/polysaccharide complexes have found a wide range of applications in different industrial sectors [1–3]. They have been used to stabilise and form structures in the food and cosmetic industries and to microencapsulate active compounds and purify proteins in the pharmaceutical and biotechnological industries [1,4,5]. One emerging interest related to protein/polysaccharide complexes is their potential application as novel emulsifies. Using different combinations, it is possible to create better and unique emulsification performance relative to that of the individual components [6,7]. This arises from the following considerations [7,8]:

(i) The complexes would have superior amphiphilicity when the largely hydrophobic proteins are associated with the hydrophilic polysaccharides.
(ii) Proteins are much more surface active and have faster interfacial adsorption kinetics than polysaccharides; the combination can provide an efficient reduction in interfacial tension and also can enhance the adsorption of polysaccharides on to interfaces to provide a better surface coverage at lower bulk concentrations.
(iii) The large hydrodynamic size of polysaccharides adsorbed on to interfaces and protruding into the water phase acts as a thick steric layer, which provides effective stabilisation between emulsion droplets and acts in concert with electrostatic repulsive forces.

Factors influencing protein/polysaccharide complexation in bulk solutions, such as pH, salt concentration, salt type, temperature, charge density and chain conformation apply similarly to that in emulsions, which control the formation, adsorption and subsequent aggregation of complexes at oil–water interfaces [7]. Among those, pH, ionic strength and protein/polysaccharide ratio are possibly the most important parameters. pH and ionic strength determine the affinity of protein/polysaccharide interaction and the complex's neutrality and therefore have a direct effect on the emulsifying behaviour and stability. The effect of protein/polysaccharide ratio is more complex. Dickinson and McClements demonstrated that at a constant protein concentration, the gradual addition of oppositely charged polysaccharide can induce the protein-stabilised emulsions going through successively four

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different states: stable (nearly no polysaccharide), unstable (bridging flocculation), stable (multilayer formation) and unstable (depletion flocculation) [6,9–11]. Moreover, the order of adding individual protein, polysaccharide or their mixtures during emulsification is also crucial, because it controls the organisation and structure of protein/polysaccharide at interfaces, creating so-called bilayer emulsions or mixed emulsions [10,12].

To better control the emulsifying functionality of protein/polysaccharide complexes, it is key to understand its relationship with the phase diagram and structures of complexes. We have previously studied the complexation of BSA with SBP and established a phase diagram in terms of pH and protein/polysaccharide ratios [13]. Detailed structural transitions of the complexes were identified. In this paper, the relationship between emulsifying functionality and the phase diagram and structures of complexes will be established using BSA/SBP system, which is expected to be applicable to other protein/polysaccharide complexes. The underlying interfacial mechanisms will be elucidated.

The practical interest is to enhance the emulsifying functionality of SBP. This relatively new type of pectin, extracted from sugar beet pulp, has received significant attention recently [14–18]. Due to different structural characteristics, SBP does not have the capability of forming gels like conventional pectins but possessing excellent emulsifying properties. The emulsifying mechanism seems to relate to the presence of proteinaceous moieties and the higher contents of ferulic acid and acetyl groups [14,17,18]. SBP-stabilised emulsions have a relatively low stability, which limits their industrial uses. The low stability may be due to its low absorption ability [15]. It was found that even at high bulk concentrations only 0.15–0.20% SBP was adsorbed on to oil–water interface [15,18]. This level of adsorption is not sufficient to provide effective steric stabilisation over long-term period and against harsh conditions. Modification by maturation technology was reported to improve the emulsion stability of SBP [16], which however, decreased its solubility significantly. SBP complexing with proteins is considered by us a possible way to solve the problem without loss of solubility.

2. Materials and methods

2.1. Materials

SBP and BSA were obtained from San Ei Gen F.F.I. Inc. (Japan) and Sigma–Aldrich (UK), respectively. The polysaccharide has a weight-average molecular weight M_w of 5.3×10^5 Da and a radius of gyration R_g of 41 nm as determined by GPC–MALLS. SBP has a protein content of 1.6% as determined by the Bradford method. BSA has a minimum purity of >96%. All other chemicals used in the study were purchased from Fisher Scientific (UK) and were of analytical grade.

2.2. Preparation of SBP and BSA stock solutions

BSA and SBP aqueous solutions (2.5 wt%) were prepared by dispersion in distilled water, followed by hydration at ambient temperature overnight on a roller mixer. The solutions were centrifuged at 4000*g* for 30 min to remove any insoluble materials. The actual concentrations of the solutions after centrifugation were determined by evaporating a known amount of the solutions at 60 °C until constant weights were achieved and weighing the dry residues. NaN_3 (0.005%) was added to prevent biological degradation of the solutions. The stock solutions were stored in refrigerator and used to prepare BSA/SBP mixed solutions with different protein/polysaccharide ratios (r).

2.3. Emulsion preparation

BSA/SBP-stabilised emulsions were prepared by reference to the phase diagram of BSA/SBP complexes established previously [13]. Two sets of conditions are used: (pH = 7.0, r = 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0) and (pH = 4.0, r = 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0). These correspond to different locations across the regions defined in the phase diagram (Fig. 1). The emulsions were prepared as follows: 40 g of 1.875% BSA/SBP mixed solutions at various ratios r were made using the above stock solutions. A 7.5 g of middle-chain triglyceride (MCT), 0.5 g of 10% benzoic acid and 2 g of distilled water were added, followed by pre-homogenisation at 26,000 rpm for 3 min using a Polytron homogeniser. The pre-emulsions were adjusted to pH 7.0 or pH 4.0 with HCl and passed twice through a high pressure homogeniser at 50 MPa. The pH of the final emulsions was checked again and fine-tuned to pH 7.0 or pH 4.0 if necessary. The final emulsions, therefore, have a total biopolymer concentration of 1.5% and a MCT content of 15%.

2.4. Evaluation of emulsion stability

There are several methods to evaluate the long-term stability of emulsions such as heating at elevated temperatures, centrifugation, shaking and stirring [19,20]. In the present study, acceleration test at 60 °C was adopted, as it was shown to provide the most accurate prediction of emulsion stability [19]. Emulsions were sealed into 20 mL glass bottles with a small headspace to minimise evaporation and were left in an oven preset at 60 ± 0.1 °C. Samples after storage for 0 day (freshly prepared emulsions), 1 day, 3 days, 5 days and 7 days were taken for laser diffraction and light microscopy measurements.

2.5. Laser diffraction measurements

Mastersizer 2000 (Malvern, UK) was used to determine size and size distributions of the emulsions. A few drops of emulsion were

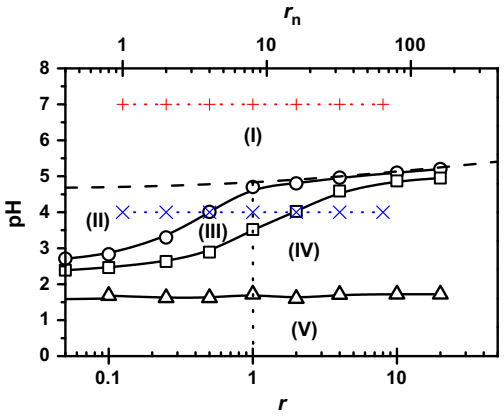

Fig. 1. Conditions to prepare BSA-/SBP-stabilised emulsions with reference to the related phase diagram that has been reported previously [13]: the first set of conditions are at pH 7.0 and different r(+) and the second set are at pH 4.0 and different r(×). (r and r_n are the protein/polysaccharide mixing ratio by weight and number, respectively). The vertical dotted line at r = 1.0 represents the maximum stoichiometry of BSA/SBP, which to the left is the SBP-excessive domain and to the right is the BSA-excessive domain. The emulsion preparation conditions, therefore, span four different regions as identified previously [13]: (I) stable region of mixed individual soluble polymers, (II) stable region of intramolecular soluble complexes, (III) quasi-stable region of intermolecular soluble complexes and (IV) unstable region of intermolecular insoluble complexes. The region (V), that is, a second stable region of mixed individual soluble polymers, is not considered in the study.