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Serum albumin forms a lactoferrin-like soluble iron-binding complex in presence of hydrogen carbonate ions

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ABSTRACT

The iron–lactoferrin complex is a common food ingredient because of its iron-solubilizing capability in the presence of hydrogen carbonate ions. However, it is unclear whether the formation of a stable iron-binding complex is limited to lactoferrin. In this study, we investigated the effects of bovine serum albumin (BSA) on iron solubility and iron-catalyzed lipid oxidation in the presence of hydrogen carbonate ions. BSA could solubilize >100-fold molar equivalents of iron at neutral pH, exceeding the specific metal-binding property of BSA. This iron-solubilizing capability of BSA was impaired by thermally denaturing BSA at $\geqslant$70 °C for 10 min at pH 8.5. The resulting iron–BSA complex inhibited iron-catalyzed oxidation of soybean oil in a water-in-oil emulsion measured using the Rancimat test. Our study is the first to show that BSA, like lactoferrin, forms a soluble iron-binding complex in the presence of hydrogen carbonate ions.

1. Introduction

Iron deficiency is a major nutritional problem worldwide, particularly among infants, school-aged children, and women of reproductive age (World Health Organization., 2008). Iron fortification of food products is a cost-effective and long-term strategy for iron supplementation (Hurrell, 2002). Accordingly, iron-fortified foods have been developed to satisfy the dietary recommendations for iron and to reduce the incidence of anemia (Hurrell, 1997). However, there are many technological problems to overcome in developing iron-fortified foods because iron is a transition metal that reacts with other components of foods, causing organoleptic defects, lipid oxidation, and sedimentation (Waraho, McClements, & Decker, 2011).

Lactoferrin, an iron-binding glycoprotein of the transferrin family, is present in milk and secretory fluids, and can bind iron at sites other than its chelate-binding sites (Baker & Baker, 2009; Nagasako, Saito, Tamura, Shimamura, & Tomita, 1993). It can solubilize 70 M equivalents of iron in the presence of hydrogen carbonate ions (Kawakami, Dosako, & Nakajima, 1993; Ueno, Kato, Ueda, Matsui, & Nakajima, 2012a). The iron–lactoferrin complex has no metallic taste, provides greater iron bioavailability than inorganic iron, and does not damage the gastric mucosa of rats (Uchida, Oda, Sato, & Kawakami, 2006). The iron–lactoferrin complex also acts as a natural iron stabilizer in a water-in-oil emulsion, and reduces iron-catalyzed off-flavor formation of powdered milk containing fish oil (Shiota, Uchida, Oda, & Kawakami, 2006; Ueno, Shiota, Ueda, Isogai, & Kobayashi, 2012b). Therefore, the iron–lactoferrin complex is a useful food ingredient for the simultaneous supplementation of a single food with iron and fish oil, by preventing anemia without the adverse effects associated with general iron supplementation.

It is of our particular interest to know whether the formation of a soluble iron–protein complex in the presence of hydrogen carbonate ions is limited to lactoferrin or whether iron could form complexes with other proteins. Serum albumin is the most abundant plasma protein in mammals. It helps to maintain oncotic pressure and acts as a carrier of nutritional substances, including steroids, fatty acids, and thyroid hormones, in plasma (Peters Jr., 1985). Bovine serum albumin (BSA), found in whey, milk, and plasma, shows high affinity to divalent metal cations, particularly copper and zinc ions, at specific binding sites. The metal-binding properties of BSA have been extensively characterized in terms of its metal-binding sites (Matsuoka & Saltman, 1994; Spector, John, & Fletcher, 1969). However, to our knowledge, no studies have reported an interaction between serum albumin and Fe^{3+} in the presence of hydrogen carbonate ions.

In the present study, we first show that BSA is capable of solubilizing iron (III) chloride at neutral pH in the presence of hydrogen carbonate ions. We then confirm that BSA forms a soluble iron-binding complex in aqueous solutions containing hydrogen carbonate ions. Finally, we demonstrate the antioxidant activity of this iron–BSA complex in a model emulsion using soybean oil.

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2. Materials and methods

2.1. Materials

Bovine serum albumin (Faction V) was purchased from Iwai Chemical Company (Tokyo, Japan). Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) was of food-additive grade and was purchased from Junsei Chemical (Tokyo, Japan). Food-additive grade sodium hydrogen carbonate, as a source of hydrogen carbonate ions, was purchased from Wako Pure Chemicals (Osaka, Japan). Bovine lactoferrin (95% purity) was obtained from Tatua Co-operative Dairy Company (Tatuanui, New Zealand). All chemical reagents were of analytical grade, unless otherwise specified.

2.2. Heat treatment of BSA

A solution of BSA (8 mg/mL) was prepared in 120 mM sodium hydrogen carbonate at pH 8.5. Aliquots (10 mL) were transferred into glass test tubes (16 mm internal diameter, 125 mm long; Asahi Glass, Tokyo, Japan), sealed with glass caps, and then heated for 10 min in a water bath at temperatures ranging from 40 °C to 80 °C. After heat treatment, the BSA solutions were immediately cooled on ice.

2.3. Preparation of iron–protein mixtures

Solutions of BSA, heat-treated BSA, and lactoferrin (8 mg/mL) were prepared in 120 mM sodium hydrogen carbonate at pH 8.5. Then, 3 mL of each protein solution was mixed with 3 mL of iron (III) chloride solution (0.625–48.4 mM) at room temperature. Samples were then stored at 0–5 °C until analysis.

2.4. Iron and protein content

The iron content was determined as described in our previous report (Ueno et al., 2012b). The soluble iron concentration in each sample was determined by inductively coupled plasma spectrometry (ICPS-8000; Shimadzu, Kyoto, Japan) after centrifuging the supernatants at 800*g* for 10 min. The free iron concentration was measured in the filtrate after ultrafiltration (Amicon Ultra; Millipore, Billerica, MA, USA; 10,000 Da cutoff). The soluble protein content in the supernatants of each sample was measured using a protein assay based on the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA).

2.5. Turbidity measurement

The turbidity of each solution was measured using an ultraviolet/visible light spectrophotometer at 600 nm (U-3010; Hitachi, Tokyo, Japan) (Ueno et al., 2012c). The samples were placed in quartz cuvettes with a cell path length of 1.0 cm. Distilled water was used as a blank reference. The cuvettes were tapped several times to ensure homogeneity before being placed in the spectrophotometer.

2.6. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of heat-treated BSA under reducing and non-reducing conditions

Heat-treated BSA (10 μg) was mixed with sample buffer (0.125 M Tris–HCl [pH 6.8] containing 2% [w/w] SDS, 30% [v/v] glycerol, and 0.002% [w/v] bromophenol blue) with (reducing conditions) or without (non-reducing conditions) 5% (v/v) 2-mercaptoethanol. The samples were boiled for 2 min to reduce the disulfide bonds and were resolved by PAGE (16% polyacrylamide) in a vertical slab gel apparatus (Safety-Cell Mini STC-808; Tefco, Tokyo, Japan) at 200 V per gel. A protein molecular weight marker (3.5–205 kDa) was included on each gel (Protein molecular weight marker II; Tefco). The gels were stained using Bio-Safe Coomassie Blue G-250 stain (Bio-Rad Laboratories).

2.7. Antioxidant assay using the Rancimat test

The antioxidant activity of the iron–protein mixtures was evaluated according to the method of Shiota et al. (2006) in the induction period using the Rancimat test (E679; Metrohm, Herisau, Switzerland) after being heated at 120 °C under atmospheric pressure. The induction time was defined as the time to a sudden increase in conductance caused by the collection in distilled water of short-chain volatile acids formed by lipoperoxidation. The iron–protein mixtures, iron (III) chloride solution, BSA, and lactoferrin were dissolved in distilled water. Then, 500 μL of each of these solutions was added to 5 g oil mixed with 3% emulsifier (Emulsy H-RO; Riken Vitamin, Tokyo, Japan). Iron was added to each solution to a final concentration of 10 ppm. The mixtures were emulsified using a desktop disperser (Ultra-Turrax T-25; Janke & Kunkel, Staufen, Germany) at 20,500 rpm for 1 min. Oil samples (3 g of each) were transferred to the reaction vessels, and air (20 mL/min) was supplied into the vessels.

3. Results and discussion

3.1. Effects of serum albumin on iron solubility in the presence of hydrogen carbonate ions

The effects of BSA on the solubility of Fe^{3+} were investigated in the presence of hydrogen carbonate ions with lactoferrin as a positive control. The soluble iron concentration was measured in mixtures containing various concentrations of iron (III) chloride, 4 mg/mL BSA, and 60 mM sodium hydrogen carbonate (Fig. 1). BSA solubilized over a 100-fold molar equivalent of iron in the presence of hydrogen carbonate ions, similar to the molecular ratio of soluble iron to lactoferrin. Fig. 2 shows the changes in color and precipitate formation in the BSA solutions containing varying concentrations of iron in the presence of hydrogen carbonate ions. The color of the iron–BSA mixture changed from yellow to red in association with increasing iron concentration, and no precipitation was observed at 100 M equivalents of iron to BSA, or less. These mixtures were stable at least 7 days because no precipitation

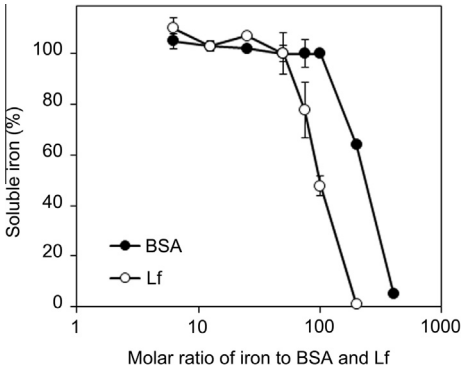

Fig. 1. Effects of bovine serum albumin (BSA, ●) and lactoferrin (Lf, ○) on iron solubility in the presence of hydrogen carbonate ions at neutral pH. BSA and lactoferrin (8 mg/mL) were dissolved in 120 mM sodium hydrogen carbonate solution at pH 8.5. The resulting solution was mixed with the same amount of iron (III) chloride solution containing 0.625–48.4 mM iron. The pH of the solutions ranged from 6.8 to 8.0. The molecular ratio of iron to BSA or Lf ranged from 6.25 to 400 (mol/mol). Data are means ± standard deviation ($n = 3$).