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Stabilization of whey protein isolate (WPI) through interactions with sugar beet pectin (SBP) induced by controlled dry-heating☆

Phoebe X. Qi [a, *], Yingping Xiao [a, b], Edward D. Wickham [a]

[a] Dairy and Functional Foods Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Wyndmoor, PA 19038, USA

[b] Institute of Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang 310021, PR China

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ABSTRACT

It has been shown previously that reacting whey protein isolate (WPI) with sugar beet pectin (SBP) through a Maillard type reaction in a dry-state led to noticeable improvement in the physical, chemical and functional properties of the end products. Studies on the changes in the molecular structure of whey protein as a result of the reaction were carried out in this work to provide a molecular understanding of these observed improvements. The effect of interacting and conjugating with SBP on the apparent secondary structure of WPI was examined by far-UV circular dichroism (CD) spectroscopy and followed by spectral deconvolution. Results showed that the structure of WPI, especially the antiparallel β-sheet element, was preserved by reacting with SBP at the weight ratios of WPI:SBP = 3:1, 2:1, and 1:1. The analysis of difference UV-VIS spectra indicated significant changes occurred in the molecular electronic states of both WPI and the feruloyl moieties of SBP. Initial Maillard reaction products were detected in the form of the FRET complexes with WPI as the donor, and SBP, the acceptor by the studies of the steady-state fluorescence and the lifetime of the excited states. The tertiary structural contacts in WPI, probed by the intrinsic Trp fluorescence, were disrupted significantly upon reacting with SBP at an equal ratio (1:1). The thermal stability of WPI, however, at both secondary and tertiary structural levels, was greatly improved by the dry-heating induced reaction with SBP at all levels. The thermal stability of the feruloyl groups of SBP was also enhanced by reacting with WPI. Furthermore, the fluorescence lifetime studies definitively identified the distinctive advanced Maillard reaction end products (AMREPs) formed between WPI and SBP at varying ratios.

1. Introduction

A natural by-product from the cheese making process, whey proteins contain all the essential amino acids and are ranked among the highest in terms of protein quality (Hoffman & Falvo, 2004). Because of their intrinsically high nutritional value as a food ingredient (Barth & Behnke, 1997), whey proteins have found numerous applications including infant formula (Jost, Maire, Maynard, & Secretin, 1999), sports nutrition (Hayes & Cribb, 2008), weight management (Pal, Ellis, & Dhaliwal, 2010) as well as clinically demonstrated uses in combating liver disease (Kume, Okazaki, & Sasaki, 2006), diabetes (Jakubowicz & Froy, 2013), and cancer (Krissansen, 2007; Marshall, 2004). In addition, whey proteins are well recognized for their superior and versatile functional properties (Bryant & McClements, 1998; Foegeding & Davis, 2011; Kinsella & Whitehead, 1989; Morr & Ha, 1993; Smithers, 2015; de Wit, 1998) such as emulsification, whipping and foaming, gelation and water holding, and are thus widely used in food products such as baked goods, salad dressings, protein beverages, and edible food packaging. Undoubtedly, these exceptional nutritional and functional characteristics of whey proteins are closely related to their molecular structures and biological functions.

The major protein components of whey are β-lactoglobulin (β-LG), α-lactalbumin (α-LA), immunoglobulins (IgG) and bovine serum albumin (BSA), and together they make up about 90% of the total whey proteins (Cayot & Lorient, 1997). It is the molecular structures and the complex interactions of these proteins with one

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* Corresponding author.
E-mail address: Phoebe.Qi@ars.usda.gov (P.X. Qi).

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another and with other ingredients in a formulation along with the effect of processing that gives rise to the nutritional and functional properties of a wide variety of food products containing whey. As commonly and widely employed by the food industry, whey proteins are still limited and excluded by certain formulation conditions and processing operations which prevent their potentials to be fully realized (Cayot & Lorient, 1997; Foegeding & Davis, 2011; Foegeding, Davis, Doucet, & McGuffey, 2002; McIntosh et al., 1998). These impediments include reduced solubility (Kazmierski & Corredig, 2003; Pelegrine & Gasparetto, 2005; Ryan & Foegeding, 2015; de Wit & Kessel, 1996), diminished emulsion stability (Demetriades, Coupland, & McClements, 1997; Fachin & Viotto, 2005; Hunt & Dalgleish, 1994; McClements, Monahan, & Kinsella, 1993; Moon & Mangino, 2004) and undesirable gelation and coagulation (Kinsella & Whitehead, 1989) over storage time and/or at elevated temperature, increased salt concentration and acidic or basic pH due to protein denaturation, polymerization and aggregation.

To overcome these techno-functional issues, recent research interest and effort have been focusing on conjugating whey proteins with carbohydrates including mono- and polysaccharides through a Maillard-type reaction (de Oliveira, dos Reis Coimbra, de Oliveira, Zuñiga, & Garcia Rojas, 2016; Dickinson, 2009, 2015; Evans, Ratcliffe, & Williams, 2013). A growing number of attempts has proved successful at improving the emulsifying and gelation properties as well as the thermal stability of whey proteins by the use of this simple non-enzymatic modification process (Akhtar & Dickinson, 2007; Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005; Jiménez-Castaño, Villamiel, & López-Fandiño, 2007; Kika, Korlos, & Kiosseoglou, 2007; Li, Enomoto, Ohki, Ohtomo, & Aoki, 2005; Liu & Zhong, 2012; Neirynck, Van der Meeren, Bayarri Gorbe, Dierckx, & Dewettinck, 2004; Rich & Foegeding, 2000; Shekaripour, Aminlari, Niakosari, & Eskandary, 2013; Spotti et al., 2014; Spotti et al., 2013; Tabatabaee Amid & Mirhosseini, 2014; Wang & Ismail, 2012; Xu, Wang, Jiang, Yuan, & Gao, 2012; Yadav, Parris, Johnston, Onwulata, & Hicks, 2010). Among these covalently conjugated systems studied thus far, it has been shown that when a simple sugar is used in the reaction, its molecular structure or type plays an important role in the improvement of the specific functional properties of whey proteins (Chevalier, Chobert, & Dalgalarrondo, 2002; Rich & Foegeding, 2000). Furthermore, past research also demonstrated that conjugation of whey proteins with the appropriate amount of polysaccharide such as dextran (Akhtar & Dickinson, 2003; Jiménez-Castaño et al., 2007; Spotti et al., 2014), maltodextrin (Akhtar & Dickinson, 2007; Martinez-Alvarenga et al., 2014), xanthan gum (Benichou, Aserin, Lutz, & Garti, 2007), carboxymethylcellulose (Kika et al., 2007; Koupantsis & Kiosseoglou, 2009) and pectin (Mishra, Mann, & Joshi, 2001; Neirynck et al., 2004; Xu et al., 2012; Xu, Yuan, Gao, McClements, & Decker, 2013) via controlled dry-heating (temperature, relative humidity and holding time) leads to a far more significant improvement in their emulsifying properties and heat stability compared to mono- or disaccharides.

Among the many polysaccharides widely used in foods, sugar beet pectin (SBP) is of particular interest because it differs from other sources of pectin in that it tends to have a higher degree of acetylation (DAc) and a higher amount of neutral sugar side chains (rich in hairy regions), making it less favorable as a gelling agent (Pippen, McCready, & Owens, 1950), unlike pectin from citrus peels or apple pomance. Instead, SBP is commonly used as an emulsifier because of its high content of the proteinaceous materials believed to be bound to the side chains through covalent linkages (Funami et al., 2007; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Siew & Williams, 2008; Williams et al., 2005). In addition, SBP is also rich in ferulic acid, both in free form (Ferreira, Diez, Faulds, Soliveri, & Copa-Patino, 2007; Qi, Chau, Fishman, Wickham, & Hotchkiss, 2014; Wicker et al., 2014) and as feruloyl groups that are ester linked to the neutral sugar side chains (Guillon & Thibault, 1990; Micard, Renard, & Thibault, 1994; Oosterveld, Beldman, Schols, & Voragen, 1996; Oosterveld, Beldman, Schols, & Voragen, 2000; Rombouts & Thibault, 1986). These important functional groups make SBP an attractive antioxidant and bioactive candidate for food and non-food applications (Graf, 1992; Kikuzaki, Hisamoto, Hirose, Akiyama, & Taniguchi, 2002; Maxwell, Belshaw, & Waldron, 2012). More importantly, the feruloyl moieties of SBP allow this class of pectin to be used as an intrinsic spectroscopic probe for studies of intermolecular interactions in complex mixtures containing multiple biopolymers such as proteins due to their distinctive absorption and fluorescence properties (Qi, Wickham, & Garcia, 2014; Silván, Assar, Srey, del Castillo, & Ames, 2011; Williams et al., 2005). To our best knowledge, many of these molecular properties of sugar beet pectin remain poorly characterized.

Despite decades of research and a vast amount of literature published, the details involved in the Maillard reaction, also known as nonenzymatic browning, including the mechanism, stoichiometry and kinetics remain elusive mainly because of the complexity of the reaction (Yaylayan & Huyghues-Despointes, 1994; van Boekel, 2001). It has been shown that the Maillard reaction involves an initial condensation of a reducing sugar with an amino group of the protein, likely the ε-amino group of the lysine, the guanidinium group of the arginine residues and the N-terminal amino group. Subsequently, a range of reactions takes place and leads, via the formation of Schiff base and the Amadori rearrangements to the formation of advanced Maillard products depending on the nature of the reactants, pH, reaction temperature and time, and humidity (Ames, 1998). Therefore, conjugation between whey proteins and polysaccharides via Maillard reaction undoubtedly causes changes in the chemical composition and physical properties such as solubility, protein conformation, and thermal stability. These changes in turn are responsible for a variety of improvements of the functional properties of the resulting conjugates. For example, it has been reported that an increased level of sulfitolysis (Klemaszewski & Kinsella, 1991) and disulfide (S—S) bonds (Monahan, McClements, & Kinsella, 1993) in polymerized whey protein isolate resulted in noticeable improvement in emulsion stability. However, an in-depth understanding of the changes that occurred in the protein at the molecular level as a result of Maillard conjugation with carbohydrates or polysaccharides is still lacking.

Moreover, the formation of a myriad of products from the Maillard reaction also complicates the identification and detailed characterization of these products at various reaction stages. The advanced Maillard reaction end products (AMREPs) have been reported in the literature (Matiacevich, Santagapita, & Buera, 2005; Morales, Romero, & Jimenez-Perez, 1996) by using the steady-state fluorescence spectroscopy. Reports are scarce or non-existent on the early stage Maillard reaction product, *i.e.* the Schiff base involving proteins and carbohydrates or polysaccharides.

In addition to conjugating whey proteins and polysaccharides by utilizing the Maillard-type reaction, successful attempts have also been made in forming complexes through non-covalent interactions such as electrostatic, hydrogen binding and hydrophobic interactions under various conditions (Alizadeh-Pasdar, Nakai, & Li-Chan, 2002; Dickinson, 2008; Evans et al., 2013; Jones et al., 2011; Mishra et al., 2001; Qi, Chau, et al., 2014). When combined, proteins and polysaccharides are expected to form either soluble complexes or coacervates depending on the conditions used (de Kruif, Weinbreck & de Vries, 2004; Schmitt & Turgeon, 2011; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003; Turgeon, Schmitt, & Sanchez, 2007). However, these complexes are transient and their practical applications are thus limited.