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Glycation of whey protein with dextrans of different molar mass: Effect on immunoglobulin E–binding capacity with blood sera obtained from patients with cow milk protein allergy

Lei Xu,* Yuansheng Gong,* James E. Gern,† Shinya Ikeda,* and John A. Lucey*‡[1]
*Department of Food Science,
†School of Medicine and Public Health, and
‡Center for Dairy Research, University of Wisconsin, Madison 53706

ABSTRACT

A growing concern around the world is the number of people who are suffering from food protein allergies. One potential approach to decrease protein allergenicity is to block IgE-binding epitopes of the protein allergen by attachment of polysaccharides via the Maillard reaction (i.e., glycation). Protein glycation has been extensively studied to modify various functional properties. We wanted to examine whether glycates could reduce IgE binding in patients with cow milk protein allergy and to explore how the size (molar mass; M_W) of the polysaccharide affects this IgE-binding capacity. Glycation was performed using the initial step of the Maillard reaction performed in aqueous solutions. The specific goal of this study was to reduce the IgE-binding capacity of whey protein isolate (WPI) through glycation with dextran (DX). Blood sera were obtained from 8 patients who had been diagnosed with cow milk protein allergy, and a composite sera sample was used for IgE-binding analysis by the ImmunoCap (Phadia, Uppsala, Sweden) method. The WPI was glycated with DX of M_W ranging from 1 to 2,000 kDa, and the M_W of purified glycates was determined using size-exclusion chromatography coupled with multiangle laser light scattering. The WPI to DX molar ratios in the glycates made from DX that had M_W values of 1, 3.5, 10 (G10), 150, 500, and 2,000 kDa were 1:4, 1:3, 1:2, 1:1.5, 1:1, and 1:1, respectively. With the increase in the M_W of DX, there was an increase in the M_W values of the corresponding glycates but a decrease in the number of bound DX. The WPI-DX glycates had lower whey protein IgE-binding capacity than native WPI, with the lowest IgE-binding capacity obtained in the G10 glycate. The DX binding ratios and morphology results from atomic force microscopy images suggested that glycation of WPI with small-M_W DX resulted in extensive protein surface coverage, probably due to the attachment of up to 4 DX molecules per whey protein. The lower IgE binding of the G10 glycate was likely due to greater steric hindrance (or a physical barrier) at the surface of the protein. In summary, our results demonstrate that glycating WPI with DX via Maillard reaction can potentially be used to decrease the allergenicity of whey protein.

Key words: dextran, whey protein isolate, Maillard reaction, cow milk protein allergy, protein-polysaccharide glycates

INTRODUCTION

Cow milk protein allergy (**CMPA**) is a common food protein allergy, especially in infants, with the reported incidence ranging from 0.1 to 7.5% globally, although the incidence significantly decreases in children older than 3 yr (Wal, 2002). Cow milk protein allergy is commonly defined as an immunologically mediated reaction that could be IgE or non-IgE associated and is triggered by a potential sensitizing antigen (protein) in cow milk. Various types of milk proteins (both caseins and whey proteins) have been implicated in this reaction (Wal, 2002). Milk-based infant formula is usually formulated with a high proportion of whey proteins. Reducing the allergenicity of whey proteins could expand their food and nutritional uses. Established dietary treatments for infants with CMPA include protein hydrolysis and the use of nondairy infant formula (e.g., soy formula; Dupont and De Boissieu, 2003). Extensive protein hydrolysis results in poor physical and functional properties as well as undesirable bitterness in the formula.

Protein functionality can also be modified through glycation, with reducing sugars or polysaccharides (for reviews, see Hattori, 2002; Liu et al., 2012; O'Mahony et al., 2016). This glycation process is usually performed by dry heating (e.g., with lyophilized samples) exploiting the initial step in the Maillard reaction. Glycation

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[1] Corresponding author: jlucey@cdr.wisc.edu

has been exploited to improve protein solubility, heat stability, and emulsification. However, dry heating processes are not feasible from an industrial perspective (de Oliveira et al., 2016). In addition, dry heating results in the formation of unwanted color and flavor compounds as well as extensive protein denaturation and aggregation. Zhu et al. (2008) developed a novel alternative method in which glycation was performed in an aqueous (wet) environment but using high concentrations of dextran (**DX**) as a crowding agent. Macromolecular crowding is the presence of high concentrations (>20%) of macromolecules in a solution; this important phenomenon is well recognized in various biological systems, such as cells (Ellis, 2001). Crowding enhances the excluded volume effect, which has the benefit of reducing the opportunity for (unwanted) protein unfolding and denaturation. With the aqueous approach, the glycation reaction can be continuously monitored by UV spectroscopy and stopped before there is unwanted color formation.

Cow milk proteins have been glycated with various sugars and polysaccharides by the dry heating method as well as chemical treatments to try to reduce their immune response (as assessed mostly by indirect methods; Hattori et al., 2000, 2004; Kobayashi et al., 2001; Bu et al., 2009; Taheri-Kafrani et al., 2009; Corzo-Martínez et al., 2010; Nodake et al., 2010; Li et al., 2011; Zhang et al., 2014). Corzo-Martínez et al. (2010) used dry heating to glycate individual whey proteins with galactose, tagatose, and small molar mass ($\boldsymbol{M_W}$) DX, but no significant reduction in IgE binding was observed for these samples. However, in that study the glycates were not purified before IgE testing (Corzo-Martínez et al., 2010). Immunoglobulin E–binding capacity is an important characteristic of food allergens and is commonly used as a measure for the evaluation of the safety of new foods, recommended in the guidance documents published by FAO/WHO (2001) and EFSA (2004) for the safety assessment of novel foods and particularly foods derived from biotechnology. In this research, the IgE-binding capacity was evaluated by the ImmunoCAP (Phadia, Uppsala, Sweden) test, one of the standard tools for in vitro allergy diagnostics in American hospitals, and approved by the US Food and Drug Administration up to a detection limit of 0.1 kUA/L (Johansson, 2004).

The goal of this research was to reduce the IgE-binding capacity of whey protein isolate (**WPI**) by glycating it with DX. Initial work by our group indicated that WPI glycated with DX (10 kDa) via the aqueous method could reduce IgE binding (Böttger, 2013). However, the effect of the M_W of DX on reducing whey protein IgE binding was not investigated. During this study, the effect of the M_W of DX, ranging from 1 to 2,000 kDa, on the properties of WPI-DX glycates was explored. The M_W of purified glycates was evaluated with size-exclusion chromatography coupled with multiangle laser light scattering (**SEC-MALLS**). Dextran has been successfully used in the past to produce individual whey protein glycates by the dry heating method (e.g., Jiménez-Castaño et al., 2007) and WPI by the aqueous method (Zhu et al., 2008, 2010). Previous studies about the effect of the M_W of polysaccharide on whey protein-polysaccharide glycates mainly focused on functionality improvements, such as emulsion stability (Dunlap and Côté, 2005), foaming properties (Ter Haar et al., 2011), heat stability (Shu et al., 1996), and other physical properties (Kato, 2002), rather than how different M_W polysaccharides affect IgE binding. There does not appear to be previous studies on the IgE-binding capacity of various sizes of whey protein glycates prepared by the aqueous heating method. Some previous studies on glycates prepared with the dry heating method did not purify glycates from the reaction mixture; thus, it was not clear whether the IgE results were actually due to glycation or could be caused by extensive protein denaturation (which can also reduce allergenicity). In the current study, purified glycates were tested by incubation with sera from blood samples obtained from patients with CMPA (as this is a more direct reflection of allergenicity than indirect chemical tests).

Our hypothesis was that glycation of WPI with DX would shield the epitopes on the allergen (whey proteins) by creating steric hindrance (physical barrier) on the protein, thus limiting the accessibility of IgE-binding sites. After characterizing the M_W, morphology, and WPI to DX binding ratio of the glycates, we evaluated the whey protein–specific IgE-binding capacity of glycates compared with the native protein (WPI). We also evaluated the microstructure and morphology of glycates by atomic force microscopy (**AFM**) to help explore the possible mechanism by which these polysaccharides may reduce IgE binding.

MATERIALS AND METHODS

Materials

For glycation we used WPI from Davisco Foods International Inc. (Le Sueur, MN), which had a total protein content of >95% (dry basis) and a lactose content of <1%. The WPI was thoroughly dialyzed against Milli-Q water (Millipore Corp., Billerica, MA) for 3 d at 5°C, with the water changed every 6 h to remove residual lactose and minerals. Dialysis membrane tubing had a M_W cutoff of 6,000 to 8,000 Da (Spectrum Laboratories Inc., Rancho Dominguez, CA). After lyophilization, purified WPI powders were stored at 5°C.