



Short communication

Ferulic acid enhances IgE binding to peanut allergens in Western blots

Si-Yin Chung*, Elaine T. Champagne

United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70124, USA

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ABSTRACT

Ferulic acid, a phenolic compound, is known to complex with proteins and peanut allergens. Preliminary studies indicated that ferulic acid could also complex with and inhibit IgE antibodies to peanut allergens in ELISA. However, results from Western blots were quite different. The objective of this study was to report the unexpected finding of IgE binding being enhanced rather than reduced by ferulic acid in Western blot. Ferulic acid, at various concentrations (0–10 mg/ml), was mixed with a pooled plasma (containing IgE antibodies) from peanut-allergic individuals before incubation with a peanut allergen-bound membrane and colorimetric detection of IgE. Results showed that an enhancement of allergen bands or IgE binding, compared to the control, was observed at a ferulic concentration of 10 mg/ml. Compounds with a similar structure, such as caffeic acid and chlorogenic acid, at the same concentration, did not have an enhancing effect on IgE binding. Tests with ferulic acid alone or soy proteins indicated that the enhanced IgE binding was due to the IgE–ferulic complexes and not ferulic acid itself. It was concluded that ferulic acid (10 mg/ml), in combination with IgE, enhanced IgE binding to peanut allergens in Western blots. The finding indicated that ferulic acid can help reduce the time for colour development of protein bands in Western blots.

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1. Introduction

Ferulic acid is a phenolic compound (Fig. 1) and an antioxidant found in many staple foods, such as fruits, vegetables, cereals, coffee, and herbs (Zhao & Moghadasian, 2008). Ferulic acid has been shown to protect against DNA damage, cancer, and other human disorders (Alias, Manoharan, Vellaichamy, Balakrishnan, & Ramachandran, 2009; Sudheer, Muthukumar, Kalpana, Srinivasan, & Menon, 2007; Zhao & Moghadasian, 2008). Because of its health benefits, ferulic acid has been approved in Japan as a food additive (JFCRF, 1996). In China, ferulic acid, in the form of sodium ferulate, is used for treatment of cardiovascular and cerebrovascular diseases (Wang & Ou-Yang, 2005). In addition, ferulic acid is a component of curcumin, the major yellow pigment in turmeric and mustard. This pigment is widely used as a food preservative and yellow agent for foods (Yang, Landau, Huang, & Newmark, 2001). Because of its prevalence in foods, intake of ferulic acid has been estimated to be at 150–250 mg/day (Zhao & Moghadasian, 2008).

Like other phenolic acids, ferulic acid is known to form soluble and insoluble complexes with proteins (Kang et al., 2004; Labuc-kas, Maestri, Perelló, Martínez, & Lamarque, 2008). It has been shown to complex with major peanut allergens and reduce the allergenic properties of peanut extracts (Chung & Champagne, 2009). Further investigations in this laboratory have indicated that

ferulic acid could also complex with immunoglobulin E (IgE) antibodies from sera of peanut-allergic patients and that, when both serum (i.e., IgE antibodies) and ferulic acid (>0.2 mg/ml) were combined and added to a microtitre plate coated with peanut allergens in an enzyme-linked immunosorbent assay (ELISA), a reduction of IgE binding to peanut allergens was observed (unpublished data). However, when a Western blot was performed in the same way (i.e., IgE mixed with ferulic acid), a different result was obtained, particularly, at a higher concentration of ferulic acid. In this study, we report the unexpected finding of IgE binding being enhanced rather than reduced by ferulic acid in Western blots. The finding indicates that ferulic acid, together with IgE, can help reduce the time for colour development of peanut allergen bands in Western blots.

2. Materials and methods

2.1. Materials

Tris buffered saline (TBS), 4-chloro-1-naphthol, Tween 20, and roasted soybean flour were purchased from Sigma Co. (St. Louis, MO). Tris–glycine pre-cast gels (4–20%), blot apparatus, and goat anti-human IgE–peroxidase were purchased from Invitrogen (Carlsbad, CA). Immobilon-P membrane was purchased from Millipore Corp. (Billerica, MA). Superblock TBS buffer and bicinchoninic acid (BCA)–protein assay kit were purchased from Pierce Chemical Co. (Rockford, IL). Human plasmas from three individuals

* Corresponding author. Tel.: +1 (504) 286 4465; fax: +1 (504) 286 4419.

E-mail address: siyin.chung@ars.usda.gov (S.-Y. Chung).

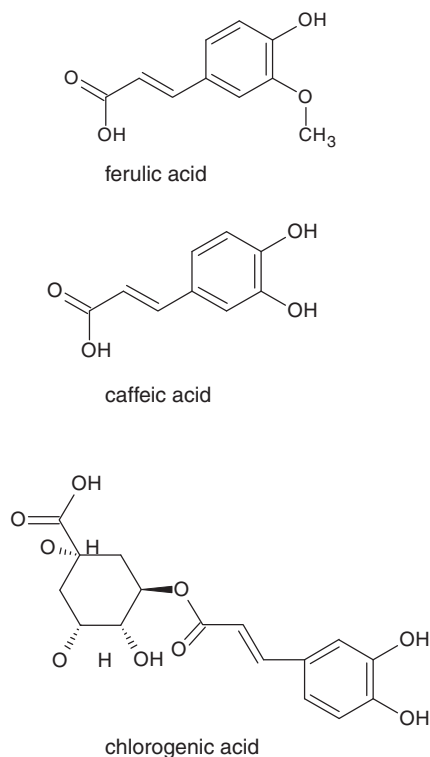


Fig. 1. Structures of ferulic, caffeic, and chlorogenic acids.

with peanut allergy (determined by CAP-FEIA assay for IgE) were obtained from PlasmaLab International (Everett, WA). Roasted high-oleic peanuts were from the University of Florida, Gainesville, FL.

2.2. Preparation of peanut and soy extracts

Peanut extracts in 20 mM Tris buffer, pH 8, were prepared from defatted roasted peanut meals, as previously described (Chung & Champagne, 2009). Soy extracts were prepared from roasted

soybean flour in the same way. Protein concentration was determined, using the BCA kit assay.

2.3. Determining the effect of ferulic acid on IgE binding in Western blots

SDS-PAGE and Western blots were performed as previously described (Chung, Kato, & Champagne, 2005) with some modifications. Briefly, peanut and soy extracts (5 μ l, 2 mg/ml) were each applied to a Tris-glycine pre-cast gel (4–20%) in SDS-PAGE, followed by transferring the proteins from the gel to an Immobilon-P membrane and blocking with a SuperBlock solution. The resultant membrane was cut into strips, and each strip was treated (rotated) for 30 min with a mixture (1:1) of ferulic acid (0–10 mg/ml) and a pooled plasma diluted 1:20 in Superblock/TBS-Tween 20 (1:1) from peanut-allergic individuals. Prior to mixing, ferulic acid was prepared in 10% dimethylformamide (DMF) in superblock/TBS-Tween 20 (1:1). After 30-min, the membrane strips were washed and incubated with a goat anti-human IgE-peroxidase (1:250) and a substrate solution of 4-chloro-1-naphthol (0.5 mg/ml) in triethanolamine-buffered saline containing 0.02% hydrogen peroxide.

3. Results and discussion

3.1. Effect of ferulic acid on IgE binding in Western blots

In this assay, a pooled plasma (containing IgE) from peanut-allergic patients was mixed with ferulic acid (0–10 mg/ml)_{final} (note: the mixture became cloudy after mixing) before adding to a membrane with bound peanut allergens/proteins. Results showed that ferulic acid at 2 or 4 mg/ml, in combination with the pooled plasma, had no effect on the colour development of protein bands on the membrane, compared to the control (i.e., 0 mg/ml) (Fig. 2). In this case, only a few and indistinct bands, corresponding to the major peanut allergen Ara h 2 (18–20 kDa), were observed after 3 min of colour development. However, when added at 10 mg/ml and combined with the plasma, ferulic acid induced the appearance of additional and darker bands on the membrane. Among these bands were major peanut allergens, Ara h 1 (63 kDa) and Ara h 3 (45 kDa), in addition to Ara h 2 [these allergens have previously

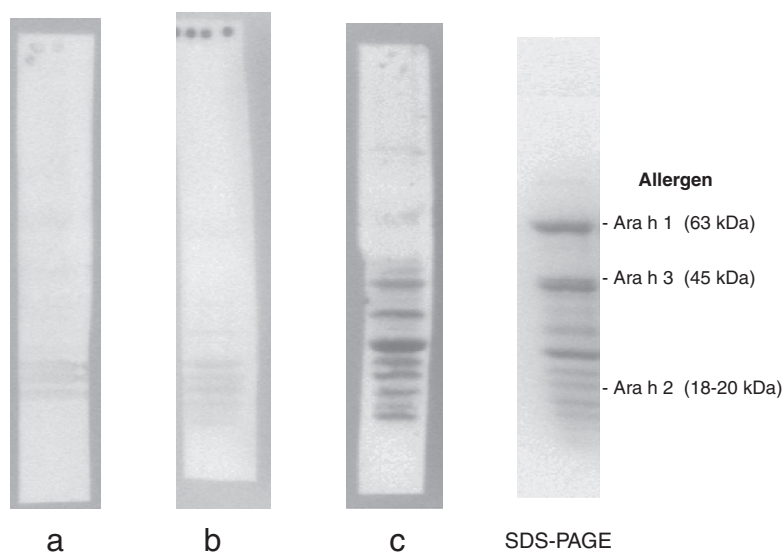


Fig. 2. Effect of ferulic acid on IgE binding in Western blots. Membranes with bound peanut proteins/allergens were incubated with a mixture (1:1) (v:v) of ferulic acid and a pooled plasma (IgE antibodies) from peanut-allergic individuals. IgE binding was detected, using a goat anti-human IgE-peroxidase conjugate and 4-chloro-1-naphthol. Final concentration of ferulic acid applied: (a) 0 mg/ml; (b) 4 mg/ml; (c) 10 mg/ml.

been purified and identified, based on IgE recognition in Western blots (Chung, Maleki, & Champagne, 2004). Other bands could be minor peanut allergens. To date, eight out of more than ten peanut allergens have been identified (Burks, 2008). The data indicate that ferulic acid at 10 mg/ml enhanced IgE binding in Western blots. However, a ferulic concentration greater than 10 mg/ml was not recommended because, at that concentration (>10 mg/ml), IgE antibodies/proteins were seen to precipitate instantly, rather than being suspended as complexes in the solution and, as a result, colour bands hardly developed.

How ferulic acid enhanced the bands in the blots is not clear. The CH₃ group in ferulic acid (Fig. 1) may be important because, when a structurally similar phenolic compound, such as caffeic acid without the CH₃ group (Fig. 1), was tested, bands on the membrane were less distinct than were those of ferulic acid (Fig. 3). A similar result (Fig. 3) was also obtained with chlorogenic acid, a larger and parent compound (Fig. 1) of caffeic acid. The binding affinity for IgE could be a factor. According to a recent study (Riihimäki et al., 2008), different phenolic compounds may have different binding affinities for the same protein. In that study, ferulic acid was shown to have approximately the same affinity for β -lactoglobulin as had caffeic acid while, in another study (Kang et al., 2004), ferulic acid was reported to show a weaker binding capacity for albumin than did chlorogenic acid. In the current study, both caffeic and chlorogenic acids behaved differently from ferulic acid on the membrane probably because they were different from ferulic acid in their degree of binding capacity for IgE antibodies. Ferulic acid probably worked by complexing with IgE antibodies and, through this process, allowing the IgE to concentrate, deposit and bind to peanut allergens on the membrane in the blot. The formation of complexes was evidenced by the cloudiness that developed after mixing of ferulic acid and the diluted plasma.

3.2. Evidence of no ferulic binding to proteins on the membrane

Ferulic binding may occur when ferulic, rather than IgE antibodies, bind to peanut allergens on the membrane and then the secondary antibody (i.e., goat anti-human IgE HRP conjugate). Another possibility is that ferulic acid, as an IgE–ferulic complex,

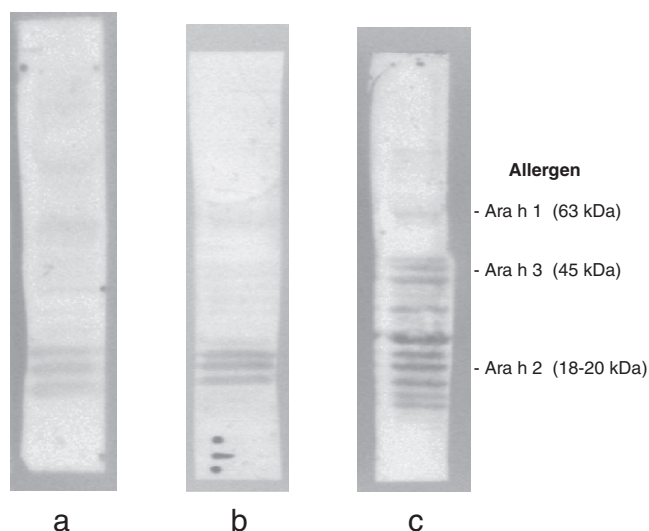


Fig. 3. Comparison of the effects of chlorogenic, caffeic, and ferulic acids on Western blots. Methods were the same as described in Fig. 2, except that the pooled plasma was mixed with: (a) chlorogenic acid; (b) caffeic acid; (c) ferulic acid (each at 10 mg/ml).

may bind to any proteins (e.g., peanut or soy). To verify that the data in Fig. 2 were not due to ferulic binding, an experiment (Expt A) was performed, in which a peanut protein-bound membrane was incubated with ferulic acid only (i.e., no plasma added). In another experiment (Expt B), soy proteins were used instead of peanut proteins, and the subsequent soy protein-bound membrane was incubated with a mixture of ferulic acid and plasma (note: same plasma from peanut-allergic individuals). A secondary antibody was finally added in both Expts A and B. The purpose of Expt A was to determine if ferulic acid itself binds to peanut allergens and/or the secondary antibody while Expt B was to determine if ferulic–IgE complexes bind to proteins (e.g., soy proteins) other than peanut allergens on the membrane. Results showed that both membranes, of Expts A and B, failed to exhibit coloured protein bands on the membranes, compared to the control (i.e., IgE/ferulic + peanut-bound membrane) (Fig. 4). This suggests that ferulic acid itself did not bind (or very little bound) to peanut allergens or the secondary antibody, and neither did ferulic acid (as an IgE–ferulic complex) bind to any of the proteins described (e.g., soy and peanut). The latter finding also indicated that the IgE antibodies were specific for peanut allergens (e.g., in the control) and not the soy proteins, or else, bands would have appeared on the soy protein-bound membrane.

Additionally, a question has been raised as to whether the result would be the same (i.e., no bands) if plasma was added (after ferulic acid) in Expt A. The hypothesis behind this was that ferulic acid may first bind to the protein-membrane and then IgE to form ferulic–IgE complexes; as a result, IgE may be blocked and not react with the secondary antibody and, subsequently, lead to no colour-band development. However, further experiments (i.e., plasma added to Expt A) did not support the above hypothesis because colour bands, rather than no bands, were observed, and the result (data not shown) was similar to the control in Fig. 2a.

In summary, using ferulic acid (10 mg/ml) in combination with a diluted serum (IgE antibodies against peanuts), in a Western blot for peanut allergens, resulted in an enhancement of IgE binding to peanut allergens on the membrane. IgE–ferulic

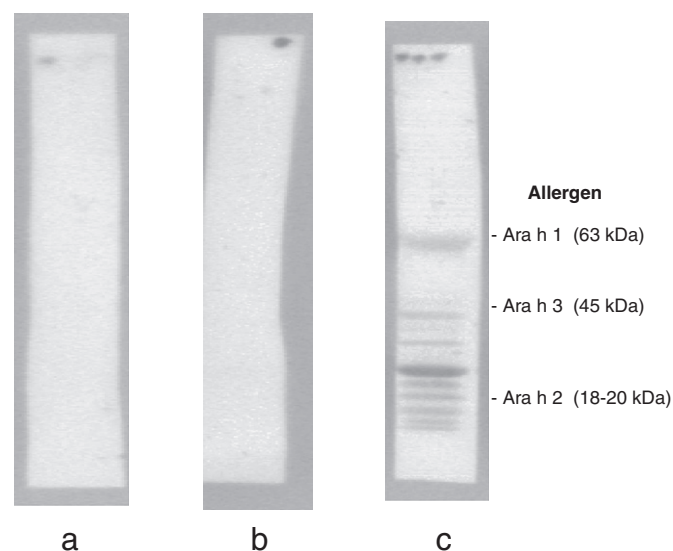


Fig. 4. Demonstration of no ferulic binding to membranes in blot assays. Membranes with soy proteins (a) and peanut proteins (c) (a control) were each incubated with a mixture of ferulic acid (10 mg/ml) and a pooled plasma from peanut-allergic individuals. In (b), membrane with peanut proteins was incubated with ferulic acid only (i.e., no plasma). After incubation, all membranes were probed with a goat anti-human IgE-peroxidase conjugate and a chromogenic substrate, 4-chloro-1-naphthol.

complex and not ferulic acid was responsible for the enhanced binding. Other compounds (caffeic and chlorogenic acids) with a similar structure at the same concentration failed to enhance IgE binding.

4. Conclusions

Contrary to our postulation that ferulic acid would reduce IgE binding to peanut allergens in Western blot, this study demonstrated that ferulic acid, in combination with IgE antibodies from a diluted plasma of peanut-allergic individuals, enhanced IgE binding or colour development of peanut allergen bands in Western blots. A ferulic concentration of 10 mg/ml was required for such enhancement. Structurally similar phenolic compounds, such as caffeic acid and chlorogenic acid, at the same concentration, did not have an enhancing effect, indicating that these compounds were different from ferulic acid in binding affinities for IgE antibodies. Tests with soy proteins and ferulic acid alone indicated that there was no ferulic, but IgE binding, to peanut allergens on the membrane. The finding indicates that, using ferulic acid, together with IgE in Western blot, can help to reduce the time for colour development of bands. Whether this work can apply to other allergens (e.g., soy and tree nuts) is not known but, because of their cross-reactivity with peanut allergens, it is possible that the serum against those allergens may work as well with ferulic acid in the enhancing of IgE binding in Western blots. Further research is still needed to verify this idea.

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