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SHORT COMMUNICATION

ICP-MS as a novel detection system for quantitative element-tagged immunoassay of hidden peanut allergens in foods

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Abstract A novel ICP-MS-based ELISA immunoassay via element-tagged determination was devised for quantitative analysis of hidden allergens in food. The method was able to detect low amounts of peanuts (down to approximately 2 mg peanuts kg^{-1} cereal-based matrix) by using a europium-tagged antibody. Selectivity was proved by the lack of detectable cross-reaction with a number of protein-rich raw materials.

Keywords ICP · MS detection · ELISA · Food allergens

Introduction

Nowadays fluorescence-based enzyme-linked immunosorbent assay (ELISA) is the "gold standard" for general screening of food samples for allergen detection [1–3]. Recently, complementary detection techniques have been developed for immunoassays [2]. Among these, inductively coupled plasma–mass spectrometry (ICP-MS) has been proposed for sensitive and quantitative element-tagged immunoassay for protein analysis in biological samples [4, 5]. Gold-cluster antibody and lanthanide (Eu, Tb, Dy, and Sm)-chelate antibody conjugates have been used to develop both direct competitive and non-competitive immunoassays, demonstrating that target human proteins can be detected at levels as low as 0.1–0.5 ng/mL and that a linear response to protein concentration over 3 orders of magnitude can be obtained [4].

ICP-MS detection offers several advantages with respect to more conventional fluorescence such as lower matrix effects from other components of the biological sample, low detection limits, excellent linearity of the response, long-term sample storage, and capability of multiple analyte detection via different element tags. However, despite its potential up to now, metal-tagged ICP-MS immunoassay has not been applied for detecting and measuring food allergens and hidden allergens, i.e., those deriving from unintentional contamination during food manufacturing. In order to assist in the control of allergen levels in foods to acceptable levels, analysts require test methods designed to produce reliable analytical information using high-throughput instrumentation.

In this context the method developed in this work combines the advantages of the non-competitive sandwich ELISA methods with the sensitivity and precision of ICP-MS detection in quantitative protein analysis via element-tagged determination. The immediate result is an innovative and accurate method for the quantitative analysis of peanut proteins as hidden allergens in foods, having the ultimate goal of multiple allergen detection. Attention was focused on the determination of the Ara h1 and Ara h3/4 peanut proteins in cereal products.

Experimental

Reagents

Phosphate-buffered saline (PBS; 150 mM NaCl, 20 mM sodium phosphate, pH 7.4), 0.01% Tween 20 in PBS, 7M nitric acid (Carlo Erba, Milan), Eu-labeled anti-mouse

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affinity purified rabbit polyclonal Abs (Delfia, Perkin Elmer Life Science, MA, USA) were used. The stoichiometry of the europium/antibody system was directly calculated in a range of Eu-labeled antibody concentrations (0.01–600 nM) by using an external calibration method with NIST-traceable europium standard solutions (High-Purity Standards, Charleston, SC, USA). The stoichiometry (10±2 Eu atoms) was found to be independent of the Ab concentration and in good agreement with that declared by the manufacturer (8 atoms of europium/antibody).

Ara h1 and Ara h3/4 purified peanut proteins and anti-Ara h1 and anti-Arah3/4 monoclonal antibodies were kindly provided by the Leibniz-Center for Medicine and Biosciences at the Research Center Borstel (Borstel, Germany). All antibody solutions were diluted with deionized water (Millipore, Bedford, MA, USA).

Instrumentation

Experimental measurements were performed on the ICP-MS X Series^{II} (Thermo Electron Corporation, Waltham, MA, USA) operating under Xt interface standard conditions. The instrument optimization was performed daily to assure a response of at least 50,000 cps/ppb for indium and 80,000 cps/ppb for uranium in the high mass range. A GS50 chromatographic pump (Dionex Corporation, Sunnyvale, CA, USA) was used to perform flow-injection analysis (FIA) with a 2% (m/v) nitric acid aqueous solution as a mobile phase delivered at a flow rate of 0.5 mL min^{-1}.

Sample treatment

Peanut and peanut-containing food extracts were prepared by adding 25 mL PBS to 1 g of ground sample. Proteins were extracted by shaking for 15 min at 60 °C, then centrifuged (14,000 rpm, 5 min) and filtered on 0.2-μm nylon filter. Milk, cacao powder, soy beans, tree nuts, rice crispies, cornflakes (all purchased in a big store), and cornflakes fortified with different amounts of peanuts were extracted by applying the same procedure.

Using the BCA test the crude extracts were analyzed for protein content that was adjusted to 1 mg mL^{-1} using PBS. Protein extracts were stored at 20 °C until analysis.

Immunoassay procedure

The assay format was based on an indirect ELISA using well-plates coated with polyclonal antibodies targeting total soluble peanut proteins. Protein standard solutions and sample extracts diluted in PBS (100 μL) were incubated for 15 min at room temperature. After washing six times with 0.01% Tween 20 in PBS, two incubation steps were performed: the first with a mixture of the mouse anti-Ara h3/4 and anti-Ara h1 antibodies and the second with the Eu-labeled anti-mouse antibodies. Each incubation step was carried out as for the sample.

After digestion with 7M HNO_3 (100 μL, 1 h, room temperature), a 25-μL sample was injected into the FIA-ICP-MS system.

To test cross-reactivity, protein extracts of protein-rich raw materials (almonds, hazelnuts, walnuts, milk, soy beans) and other food products (cacao powder, cornflakes, rice crispies) commonly used in the food industry were analyzed.

Results and discussion

The non-competitive sandwich ELISA ICP-MS assay is based on the determination of the element used to tag the antibody. The assay was constructed using polyclonal immunopurified antibodies as capture reagents and anti-Ara h1 and anti-Ara h3/4 monoclonal antibodies (Ab I) for identification. Detection was performed with europium-labeled rabbit anti-mouse polyclonal antibodies (Ab II).

In the assay development, various parameters, such as Ab concentration, incubation time, and temperature, were shown to have a significant effect on method sensitivity.

Ab I and Ab II concentrations were optimized as a function of the assay, since the results showed that the dynamic range of this method at low concentrations of antigen is limited by non-specific binding. In fact, by increasing both reagent concentrations (Ab I from 20 to 200 μg mL^{-1}; Ab II from 0.5 to 10 μg mL^{-1}) and incubation times from 15 min up to 1 h, Eu signal increased significantly both when positive and negative controls were analyzed. Incubation was carried out at room temperature and at 37 °C, but not significant differences were observed in terms of Eu signal intensity. These findings suggested that in order to detect very low amounts of antigen it is necessary to maintain a low background of Eu and a low amount of proteins that can form non-specific binding to the anti-Ara h1 and Ara h3/4 antibodies. Hence, optimal conditions were those obtained using the highest amount of antibodies tested and the lowest incubation time at room temperature.

To determine method performance, peanut extracts containing known protein concentrations in the 3–25 ng mL^{-1} range were preliminarily tested. The results showed an excellent linearity (Fig. 1a) and the detection limit of the immunoassay method (determined as 3 times the blank standard deviation/slope of the calibration curve) calculated for raw peanut protein extracts was determined to be 1.5 ng mL^{-1}. It is to be noticed that this value is directly affected by the binding affinity of the antibodies and by the non-specific background, since the ICP-MS

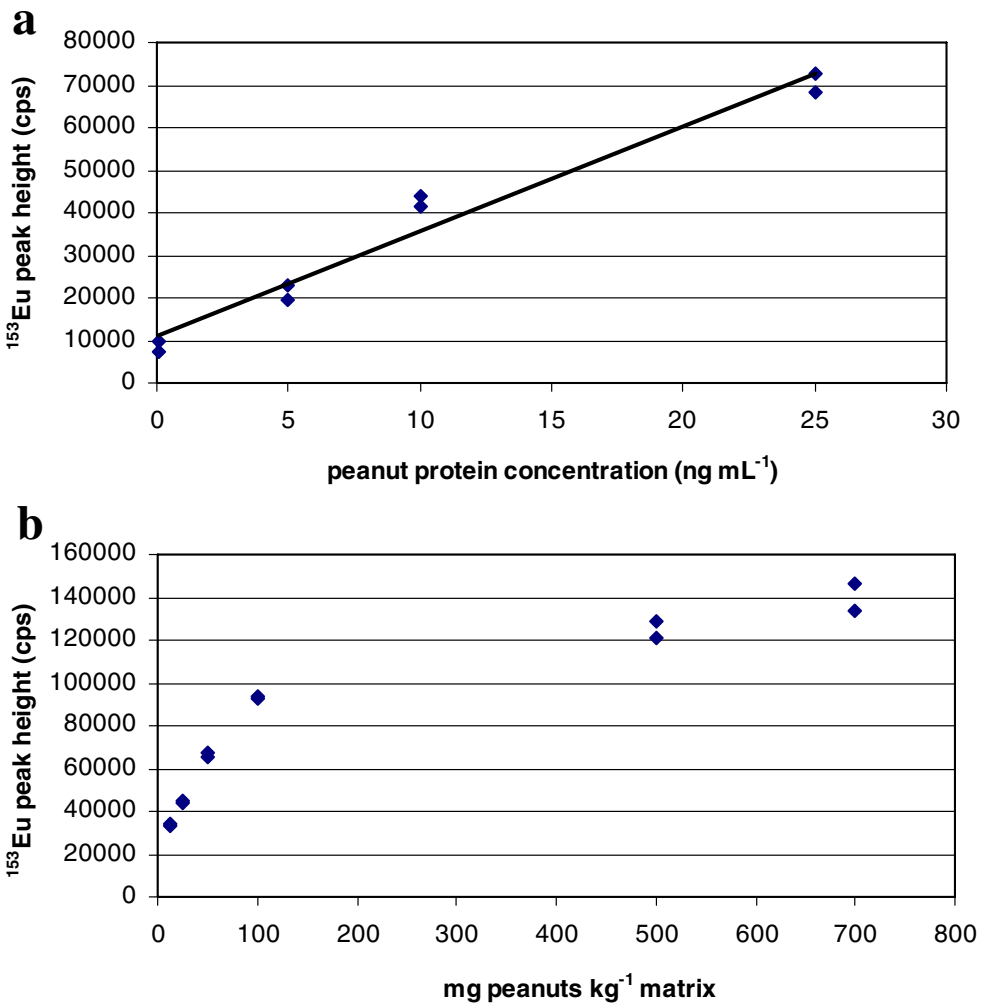

Fig. 1 ICP-MS ELISA-immunoassay calibration curves obtained by using **a** peanut proteins and **b** cornflakes fortified with different amounts of peanut

instrumental detection limit for Eu-labeled Ab was as low as 0.1 ng mL^{-1}.

The ELISA ICP-MS method developed was then evaluated in matrix for linearity, detection limit, and selectivity using known concentrations of peanut in cornflakes. Peanuts could be detected in foods down to approximately 2 mg peanuts kg^{-1} matrix. Good linearity was demonstrated by using matrix matching samples obtained by fortifying cornflakes with peanut in the range of 10–100 mg peanuts kg^{-1} matrix (r^2 =0.998, n=10) (Fig. 1b). By exploring the linearity up to 700 mg peanuts kg^{-1} matrix, a saturation of the europium signal (corresponding to an Eu-labeled Ab concentration of 90 μg mL^{-1}) was observed (Fig. 1b). Such findings indicate the limit of the antibody binding capability, since the response of the free in-solution Eu-labeled Ab was proved to be linear over the 2 ng mL^{-1} to 100 μg mL^{-1} range.

As for selectivity, the results obtained did not exhibit a detectable cross-reaction, demonstrating that the monoclonal antibodies used are very specific toward peanut proteins considering the food products reported in the "Experimental". Further investigation on the cross-reactivity will be carried out on other tree nuts and cereals.

Taking into account that commercially available tests for peanut allergens have detection limits ranging from 0.1 to 2.5 mg peanuts kg^{-1} matrix and that generally linearity is established in a narrower range [6], the ICP-MS-based method proposed is very promising, since it could guarantee adequate detection limits coupled with an improved linearity and reduced matrix effects.

Conclusions

The capability of ICP-MS as a detection system for ELISA immunoassay in food analysis was proved. In particular, a selective and sensitive immunoassay able to detect low amounts of peanuts by using an Eu-tagged antibody was devised. The described immunoassay can be used as a quantitative tool to detect peanut proteins as hidden allergens in foods. In agreement with literature data [4, 5], the bottleneck of the resulting application was the immu-

noassay protocol due to the non-specific reactivity of the reagents and lower detection limits could be reached by improving reagent quality.

ICP-MS represent a powerful detection system from several points of view and namely in terms of sensitivity and precision of the quantitative data. Future perspectives are to exploit the capabilities of ICP-MS to perform multi-element analysis by tagging directly the primary antibodies with different lanthanides and to develop a multi-tag ELISA assay able to simultaneously detect different allergens in food samples.

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