

Identification of hidden allergens within foods

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The problem of the detection of “hidden” allergens in food is a major concern for both the industry and consumers at present. The production of specific antibodies against two such food components, peanut and sesame, is described. Immunoassays are presently the method of choice for detection and identification of a wide range of food components, and ELISAs (enzyme-linked immunosorbent assays) for both peanut and sesame have been set up. Who might use such assays for maximum benefit, and in what format they should be provided, are key questions for food analysts, and the issues are discussed.

Key words: allergens; antibodies; antibodies, monoclonal; enzyme-linked immunosorbent assay; immunoassay.

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Though recognition and understanding of allergy to food components goes back centuries, interest in the detection of allergens in foods is more recent. As the food chain has become more complex, involving international dimensions and large markets, so have the potential health and economic consequences of food safety issues also increased. For the food industry, the ability to detect “hidden” allergens (that is, potentially allergenic material that should not normally be present in that food) in their products has economic consequences if products have to be recalled from the market. For the sensitized consumer, there may be direct health effects (even death) from accidental consumption of allergens, and a much wider population of consumers can exert indirect economic effects through suspicion of the industry and its ability to control food production in a safe manner. Problems of “hidden” allergens in food occur because of

- 1) the unsuspected but legitimate presence of a food material that is not declared on the label
- 2) accidental contamination of a raw material or product, either in production or during cooking
- 3) misclassification of a raw material
- 4) contamination caused by economic adulteration of raw materials and products.

For some of these problems, solutions can be achieved by greater awareness by both the industry and the consumer (indeed, much progress has been made in the last few years). For others, the

methods of analysis is critical. For the most part, “hidden allergens” are derived from plant sources; other food allergens – shellfish, for example – are central food components and their presence is usually obvious.

Individuals sensitized to a single food can differ in their response to components of that food; they can be allergic to one or more different components. Consequently, the most significant landmark of progress is to be able to detect the individual food, such as cow's milk, peanut, or sesame. The methods of choice are, in practice, limited to antibody-based analytic procedures. Such methods have a demonstrated ability for cost-effectiveness associated with specificity and sensitivity. In our studies, we have produced polyclonal and monoclonal antibodies against proteins characteristic of particular allergenic foods, and used these to develop robust enzyme-linked immunosorbent assays (ELISAs) for the detection of “hidden” allergens in foods.

Material and methods

Production of antibodies

Purified conarachin from peanut, a 7S globulin making up around 7–10% of the seed's protein, was prepared as previously described (1) and used to immunize rabbits for production of polyclonal antibodies by standard methods. The 13S globulin

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filtration (Brett & Morgan, unpublished data) and used to raise rabbit polyclonal antibodies as for the conarachin.

Immunoblotting methods

Immunoblotting was performed as previously described (2). Briefly, legume and nut samples were defatted, suspended in sample buffer, and sonicated before boiling. SDS-PAGE was performed with a 14% separating gel. Immunoblots were developed with an anti-rabbit IgG antibody labelled with alkaline phosphatase and a nitroblue tetrazolium/BCIP substrate.

ELISA methods

For antiserum titre curves, the appropriate purified protein was adsorbed onto the surface of a microtitration plate by standard procedures. Serial dilutions of putative antisera were incubated with the solid phase. After washing, bound antibody was detected with a horseradish peroxidase-labelled anti-rabbit IgG antibody. The substrate was based on TMB (3,3',5,5' tetramethylbenzidine). For standard curves and assays, the appropriate dilution of antiserum was incubated with standards/samples in a competitive ELISA format. Detection of bound antibody was as before.

Results

High-titre polyclonal antibodies against conarachin and sesame were obtained from rabbits. The antisesame antiserum could be used at dilutions of 1:50 000. The anticonarachin antiserum could be used at 1:100 000. Immunoblots (not shown) indicated that both antisera were specific, and this was confirmed when cross-reactions were determined by ELISA.

Discussion

Antibodies are able to pick out their targets in complex mixtures, and to do so with high affinity and specificity. Such ability is particularly powerful when it is required to detect individual proteins which do not have intrinsic, unique activities by which they might otherwise be recognized. In addition, antibodies can be used in formats which are simple to use, which require no expensive equipment, and which can be used for large sample numbers and batch processing or for rapid analysis of single samples. The assays can be formatted in quantitative and semiquantitative mode, including dipstick-type assays (1). Once available, the antibodies, and

provide additional information on structure, stability, and interrelationships with other proteins (2-4).

A number of immunoassays for food components that are potential allergens have now been described, though not all have been applied to allergen detection. Examples include peanut (1, 5), soya (4), wheat gluten (6), egg (7, 8), cow's milk (7, 8), and now sesame. It seems increasingly likely that the range of assays available commercially as diagnostic kits for the detection of allergens in food will increase considerably. At present, there are two kits commercially available for detection of peanut (Cortecs Diagnostics, UK; Pro-Lab, Canada). It is our view that only such kits should be used by the industry, except in those cases, such as coeliac disease, where the consequences of error are not life-threatening. Individuals are not in the position of being able to obtain a representative sample from a plate of food, and, if in doubt, the answer must be to avoid suspect foods. Consumers will be best served if the industry can label products reliably and informatively, can check raw materials and products for authenticity and contamination with appropriate methods of analysis, and can organize production such that opportunities for contamination are minimized.

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