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Chemical Composition and Structure of Food Constituents: Defining Allergenic Potential

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Introduction

Six laboratories are collaborating on a project to identify key potentially allergenic peptide sequences in selected food proteins. Studies have been initiated using β -lactoglobulin (β -LG) as the model protein because its molecular structure has been extensively documented. Within the project both sera from subjects sensitized to β -LG and specific T-cell clones (TCC) obtained from normal and atopic donors in response to cow's milk allergen are being utilized in order to obtain a more precise definition of selected peptides. Additionally an experimental animal model which provides specific β -LG IgE antibodies has been established to enable determination of the reactive sequences in β -LG that are recognized by rat sera and allow a comparison with responses found in human sera to be encoded. Methodology is in place for determining linear epitopes within β -LG, putative immunogenic peptides have been synthesized and the molecular characterization of β -LG hydrolysates generated from a range of commercial food grade enzymes has been carried out. Also in place is an intact animal model to investigate the passage of milk proteins and peptides across the intestinal mucosa. Together, these studies, using a number of different approaches, have provided the beginnings of a database for defining allergenic epi-

Table 1. Summary of Pepscan linear epitopes for rabbit serum (150 PINs)

PINs with sequential overlapping β -LG 12-mers	Common β -LG sequences
1–6	⁶⁷ TMKGLDI ¹²
21–31	³⁰ SLLDAQSA ³⁷
34–42	¹² YVEE ⁴⁵
44–50	⁵⁰ PEGDLE ⁵⁵
61–67	⁶⁷ AQKKII ⁷²
80–85	⁸⁵ DALNENK ⁹¹
102–108	¹⁰⁸ ENSAEP ¹¹³
119–128	¹²⁷ EVDD ¹³⁰
130–134	¹³⁴ EKFDKALF ¹⁴¹
146–151	¹⁵¹ FNPTQLE ¹⁴¹

topes and the immunodominant T-cell epitopes of milk allergens. Some of the preliminary results are presented in the following pages.

Materials and Results

Initial method development to identify the immunoreactive regions of β -LG has utilized sera from rabbits immunized with β -LG as only small volumes of human or rat sera were available at the start of the project. This allowed preliminary experiments to ascertain whether within the rabbit IgG response antibodies to shorter peptide lengths of β -LG were present and carried out in the knowledge that if no antibody responses occurred switching to IgE antibodies would be unlikely. Pepscan technology was used to investigate, in collaboration with Prof. R. Meloen, Pepscan Systems, The Netherlands, which linear epitopes would be recognized by rabbit anti- β -LG. Overlapping 12-mers were synthesized on a set of 150 PINs and a standard ELISA used to locate which of these peptides bound the antibodies under investigation. Pepscans obtained from six different rabbit antisera showed that the antisera tested contained antibodies which bound to a large number of synthesized 12-mers and the patterns obtained indicated that the polyclonal antibody response induced in each rabbit recognized a similar set of linear epitopes within β -LG (table 1). Experiments to give more information on similarities and differences between β -LG epitopes recognized by IgG antibodies from different species, e.g. rabbit, mouse, rat and human, are now being undertaken. Another approach has been to try to define and predict IgE epitopes by means of chemically synthesized peptides. Putative immunogenic peptides from the β -LG sequence were selected on the basis of antigen prediction, literature reports of antigen recognition and turn structure. These have been successfully synthesized using fluorenyl methoxy carbonyl chemistry on the Novabiochem synthesizer (table 2). One recent report has confirmed that three of the selected peptides may correspond to human IgE epitopes [1].

A sequence spanning residues 21–40 of β -LG is also being synthesized, as a tryptic fragment spanning these residues was shown to be recognized by human sera through a new

Table 2. Predicted immunogenic sites in β -LG

Peptide	Sequence ^a
1	³¹ LLDAQSAPLRVYEE ⁴⁵
2	³⁹ LPVYVEELKPTPEG ⁵¹
3	⁵⁴ LEILLQKWENGEC ⁶⁷
4	⁹¹ KVLVLDTDYKKYLL ¹⁰⁴
5	¹⁰⁷ MENSAEPEQSLACQ ¹²⁰
6	¹⁴⁵ RLSFNPTQLEEQC ¹⁶²
C1	¹¹ DIQK VAGTWYSLAM ²⁴
C2	¹²⁰ QCLVRTPEV DDEALEK ¹³⁵

^a Immunogenicity of sites proposed on the basis of Jamieson-Wolf antigenic data; literature reports of antibody recognition; location at loops on surface of β -LG as β -turn; location at loops on surface of β -LG as other types of turn.

ELISA test procedure. This improved ELISA procedure is an adaptation of a procedure where the immobilized antigen is incubated with human sera, followed by the addition of acetylcholinesterase-labelled anti-human IgE antibody, and the amount of binding detected by Ellman's reagent [2]. Because a peptide might be buried among the much larger protein molecules used as a saturating agent (i.e. albumin) the NH₂ groups on the peptide were biotinylated, and chromatographic separation used to obtain peptides labelled with only one biotin, located at the N-terminal amino acid residue. The purified peptide can then be immobilized under a more antigenic form, allowing a higher signal (fig. 1–4). This development for a more efficient presentation of the peptide will now be used when screening selected peptides for binding to allergenic human sera. The acetylcholinesterase assay has also been adapted for use with allergic rat sera as the Brown Norway rat has been shown to develop specific IgE antibodies after immunization with β -LG or semi-skimmed milk [3]. Interestingly the rat immune system responded to much lower concentrations of β -LG when seen in the context of milk as compared with administration of protein alone. This 'adjuvant' effect of milk will be explored during the course of the project, and when comparing information on peptide sequence responses found in rat and human sera.

Finally, TCC have been derived from peripheral blood lymphocytes of normal donors as well as atopic and milk-allergic subjects in order to investigate the mechanism of allergy induction. All subjects had memory T cells to the soluble milk proteins used as allergens and analysis of T-cell lines specific for the allergens showed no significant differences between normal and allergic donors. However, analysis of TCC derived from the cell lines revealed a significant ($p < 0.001$) lower proportion of CD8⁺-specific TCC generated from patients with severe atopy than in nonatopic subjects. Assessing the effect of selected peptides on both T-cell lines and TCC, may lead to the definition of immunodominant T-cell epitopes and a better understanding of the pathophysiological mechanisms of oral tolerance.

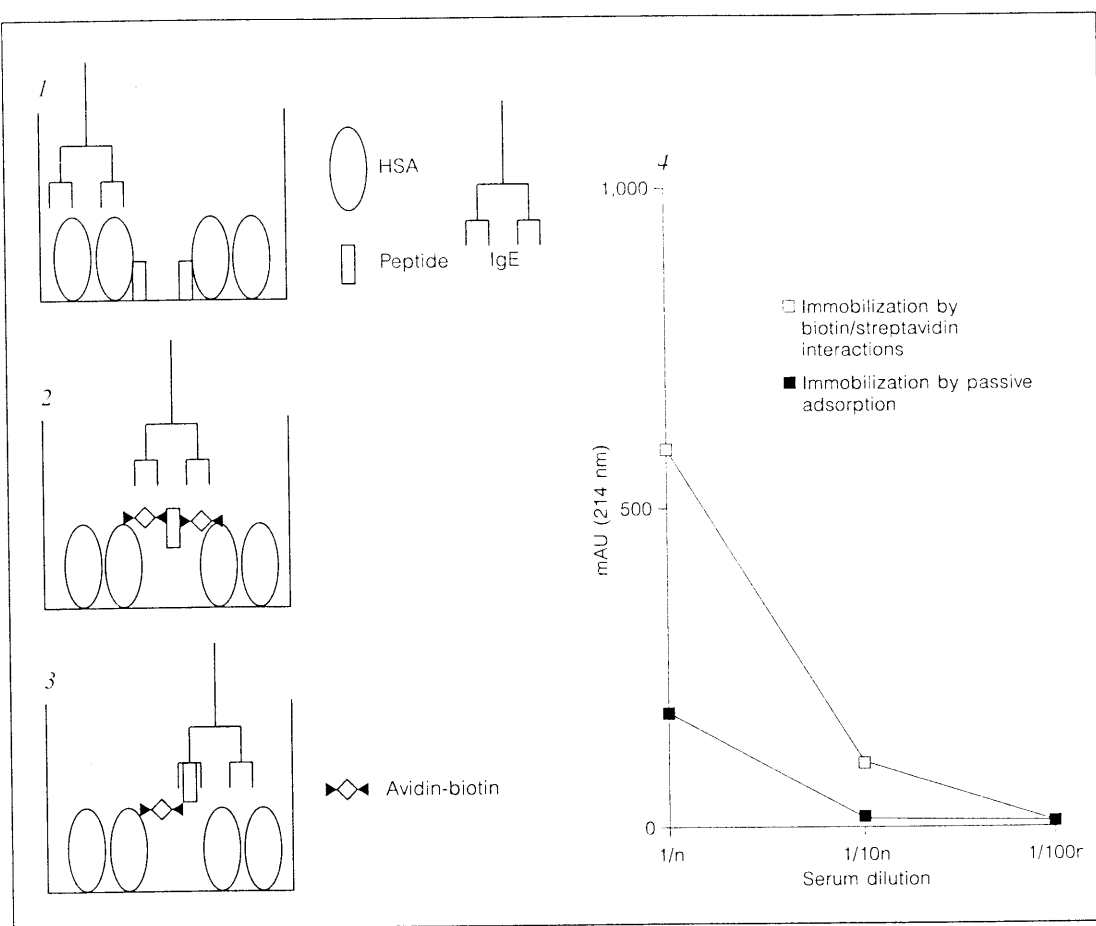


Fig. 1. Diagram demonstrating that in a direct ELISA test a peptide might be obscured by a much larger molecule (i.e. HSA) used as a saturating agent, and therefore the antigenic sites would not be accessible to IgE antibodies in solution.

Fig. 2. Diagram demonstrating inaccessible antigenic sites as a consequence of the interaction of the coupling reagent along the peptide chain.

Fig. 3. Diagram demonstrating that modifying biotinylation conditions allows greater accessibility of antigenic sites.

Fig. 4. Figure demonstrating that immobilizing a peptide under a more antigenic form results in a higher signal.

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