



IgE Epitope Mapping of the Cashew Nut Allergen Ana o 3

Introduction

In this application note, we describe the mapping of IgE epitopes of the cashew nut allergen Ana o 3 using human sera.

Peptide microarrays are the method of choice for epitope mapping. They enable the in-depth identification and characterization of antibody epitopes. PEPPERPRINT uses a peptide laser printer to produce peptide microarrays by direct synthesis of 3D amino acid chains on chip. The advantage of this technology is the huge diversity of peptide sequences that can be synthesized in parallel, the low material consumption and, hence, lower costs.

The peptide laser printer contains 24 printing units, which are filled with amino acid toner particles. During printing, a polymer-coated glass slide passes all printing units consecutively and the different amino acid particles are deposited on the position, where they are required (Fig. 1). Subsequently, the particles are melted, which releases the activated amino acids. The peptide bond can be formed with either the linker or the previous amino acid that was printed on the same position. After washing and deprotection of peptide N-terminus, the next amino acid pattern is printed and coupled. Thereby up to 100K different peptides in

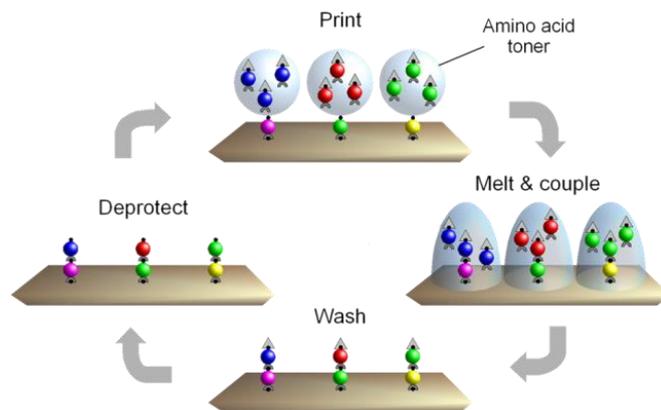


Fig. 1: PEPPERCHIP® Platform Technology: One layer of an amino acid particle pattern is printed on a coated glass slide. A heating step melts the particles and embedded amino acids can couple to the chip surface. A cycle of synthesis is completed after removal of excessive material by washing and cleavage of the N-terminal Fmoc protection group. The cycle is repeated until the full peptide microarray is synthesized.

duplicate consisting of up to 20 amino acids are synthesized in parallel. The huge sequence variety combined with low synthesis costs is unique and allows synthesizing large custom peptide libraries for e.g. serum biomarker screens. In addition, due to the digital printing flexibility custom PEPPERCHIP® peptide microarrays can be manufactured and supplied in any quantity from single microarrays to hundreds of chips within only 3 weeks and the peptide content can be easily adapted from one to the next project.

The printing flexibility also allows synthesizing overlapping peptides with a shift of one amino acid. A shift of 1 is indispensable for precise epitope mapping because the peptide shift influences

- the readout of the exact identification of conserved core motifs
- the detailed analysis of epitope length



- the unambiguous itemization of adjacent epitopes in polyclonal samples
- the discrimination of unspecific binders from real epitopes

For detailed information please download the application note: “Comparison of High Resolution PEPperMAP[®] Epitope Mappings with Low Resolution Epitope Mappings” from our website.

IgG and IgM antibodies are very abundant in human serum, making epitope mapping of IgG and IgM antibodies very easy. Since the IgE titer is about 100,000 times lower, IgE epitope mapping is much more demanding and requires a highly sensitive detection technology.

To demonstrate the potential of PEPperCHIP[®] high density-peptide microarrays for IgE epitope mapping, we chose the cashew-allergen Ana o 3 as model-protein. Ana o 3 is a 2S albumin, and one of the major allergens from cashew (*Anacardium Occidentale*). This 16 kDa protein consists of a small and a large subunit, preceded by a leader peptide [1] which is expected to be cleaved off during post-translational modification of the protein. On denaturing SDS-PAGE, Ana o 3 displays three protein bands of 6, 8 and 10kDa [1]. The serum of a cashew-allergic patient was checked for presence of Ana o 3-binding IgE by western blot prior to epitope mapping. The cashew-specific IgE level of the patient serum was determined at 33.8 kU/L.

To characterize the antibody response against Ana o 3, a high resolution epitope mapping with 15mer peptides and a peptide-peptide overlap of 14 amino acids (shift 1) was performed by PEPperPRINT. Peptides were printed in duplicate and tested with (1) serum of a patient, allergic to cashew nut, and (2) serum of a non-allergic individual. We identified several IgE-reactive epitopes with high signal-to-noise ratios in the patient sample.

Results & Discussion

Two human sera were tested for IgE responses against the cashew nut protein Ana o 3.

Cashew nut extract was loaded on an SDS-PAGE gel. Proteins were separated by their size and blotted on nitrocellulose membrane. After incubation of the membrane with human serum (1:5), rabbit anti-IgE antibody and alkaline phosphatase conjugated anti-rabbit IgG antibody, the membrane was stained with BCIP/NBT substrate. We detected IgE antibodies against cashew nut protein Ana o 3 in the serum of a patient, allergic to cashew nut. Incubation with a negative control serum sample did not give a signal (Fig. 2).

[1] Robotham, J. M., Wang, F., Seamon, V., Teuber, S. S., *et al.*, Ana o 3, an important cashew nut (*Anacardium occidentale* L.) allergen of the 2S albumin family. *The Journal of allergy and clinical immunology* 2005, 115, 1284-1290.

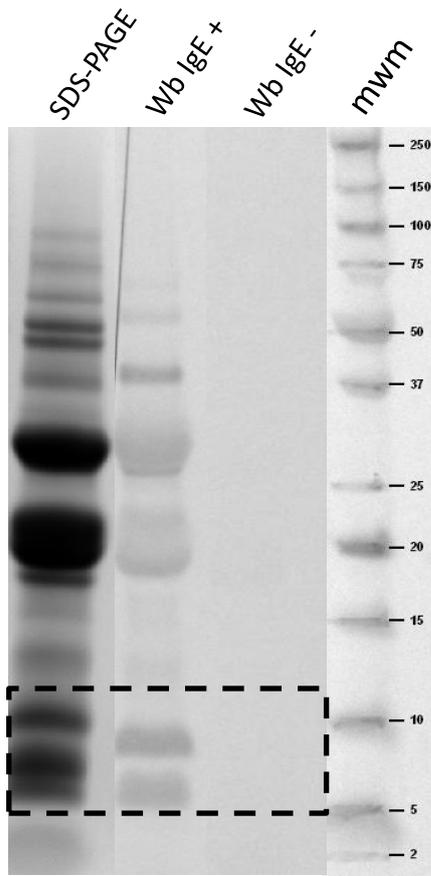


Fig. 2: SDS-PAGE and western blot of a denatured cashew extract containing, amongst others, Ana o 3 with subunits framed. From left to right: SDS-PAGE cashew extract, western blot (wb) with IgE positive serum, western blot with IgE negative control, molecular weight marker (mwm).

Cashew nut protein Ana o 3 was translated into 15mer amino acid peptides with a peptide-peptide overlap of 14 amino acids (shift 1). The resulting peptide microarrays comprised 138 different peptides printed in duplicate and were framed by HA(YPYDVPDYAG) positive control peptides.

Two peptide microarrays were incubated with human serum samples (1:2, corresponding to a total serum amount of 100 μ l per assay). Both arrays were subsequently stained with the secondary antibody goat anti-human IgE DyLight800 (1:500) in the presence of the anti-HA control antibody. Signals were detected with a LI-COR Odyssey Imaging System and spot intensities were quantified using the PepSlide[®] Analyzer software. While no IgE response was detectable in the serum of the negative control, serum of the IgE positive patient showed clear and well-defined binding to Ana o 3 (Fig. 3).

Signals from epitope mapping can be attributed to two different sets of peptides: the more dominant signals come from peptides sharing (R,Q)xC as C-terminal ends: QRQFEEQQRFRNC, RYNQRQESLREC, CQELQEVDVRR, RELYETASELPRIC and ELPRICISPSQGC. The second, weaker signals show (R,Q,K)QE C-terminal motifs: EQQRFRNCQRYVKQE, QEVQRGGRYNQRQE and CQNLEQMVRQLQQQE (Fig. 4).

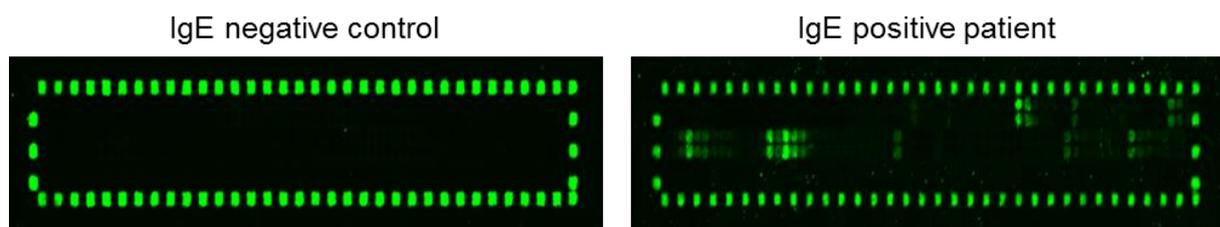


Fig. 3: Peptide Microarray Scans: PEPPERCHIP[®] Peptide Microarrays displaying cashew nut protein Ana o 3 translated into overlapping peptides. The arrays were assayed with human serum (1:2) followed by staining with labeled anti-human IgE and anti-HA antibodies. While the IgE negative control serum did not show any signal, the IgE positive patient serum exhibited clear epitope stretches. HA positive control peptides framing the peptide array showed the expected well-defined spot pattern.

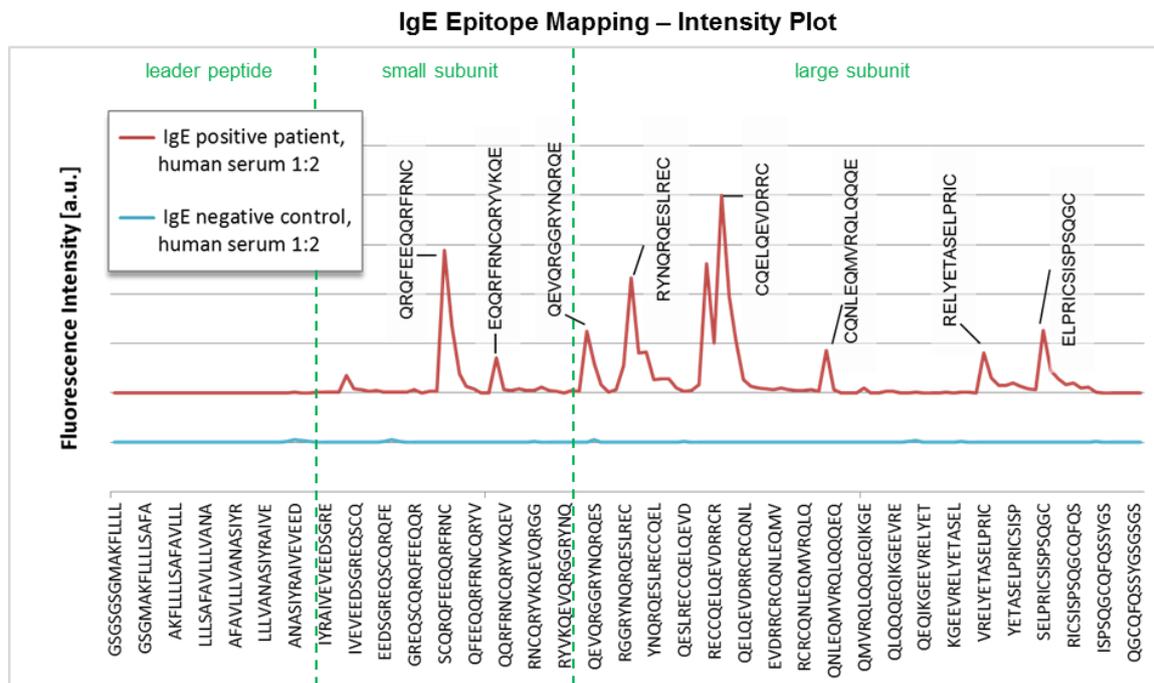


Fig. 4: Intensity Plot: IgE Epitope Mapping data of two human serum samples against cashew nut protein Ana o 3 was quantified and translated into intensity plots. We detected clear signals in the serum of the IgE positive patient that were - in a first view - attributed to two different sets of peptides. The first, more dominant signals, have (R,Q)xC as C-terminal ends: QRQFEEQQRFRNC, RYNQRQESLREC, CQELQEVDRRRC, RELYETASELPRIC and ELPRICSISPSQGC. A second, weaker set has (R,Q,K)QE as C-terminal motifs: EQQFRNCQRYVKQE, QEVQRRGGRYNQRQE and CQNLEQMVRQLQQQE. Serum of the IgE negative control did not give a signal.

Conclusion

Serum of a patient, allergic to cashew nut, was analyzed and IgE epitopes of the protein Ana o 3 were mapped and compared to a negative control serum. Despite the low concentration of IgE antibodies in human serum, IgE antibodies in the patient serum clearly bound to Ana o 3 derived peptides. IgG antibodies against the Ana o 3 antigen were not detected (data not shown). IgE signals can be attributed to two different sets of peptides, sharing either (R,Q)XC or (R,Q,K)QE motifs as C-terminal ends. A serum dilution of 1:2 was used for IgE epitope mapping, corresponding to 100 μ l serum per test, which is a fraction of the serum amounts typically required for western blot or dot blot analysis. Even though the serum was only little diluted in the peptide microarray assay, no background due to unspecific binding was observed.

Thus, with PEPperCHIP[®] Peptide Microarrays, it is possible to obtain detailed knowledge of IgE epitopes in human serum that could be used for e.g. allergy diagnostics development. The peptide printing technology invented by PEPperPRINT allows synthesizing overlapping peptides with a shift of one amino acid directly on chip. This feature is the basis for a cost effective, customizable and fast solution for high resolution IgE epitope mapping.

This application note has been generated in collaboration with M. Reitsma and S. Bastiaan-Net, Wageningen UR Food & Biobased Research, The Netherlands