

Allergenic compounds on the inner and outer surfaces of natural latex gloves: MALDI mass spectrometry and imaging of proteinous allergens[†]

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Natural latex gloves are the cause of a severe health problem to an increasing number of healthcare workers or patients due to the presence of protein allergens as Hevein or Rubber Elongation Factor (REF). One of the most challenging problems is the *in situ* localization of these allergens in, e.g. gloves, to estimate the allergenic potential of the latex material. A sample preparation protocol applying a binary matrix-assisted laser desorption/ionization (MALDI) matrix containing α -cyano-4-hydroxy cinnamic acid (CHCA) and 2,5-dihydroxy benzoic acid (DHB) on trifluoro acetic acid (TFA) etched latex glove surfaces allowed the direct determination (exact molecular weight) of Hevein, REF and a truncated form of REF (tREF) within nine different brands of natural latex gloves by means of MALDI-TOF-MS in the linear mode. MALDI mass spectrometry demonstrated that Hevein, tREF and REF were present on the inner surfaces (in direct contact with the skin) of many, but not all, investigated gloves without any prior extraction procedure. Additionally, different isoforms of the allergen Hevein were detected (exhibiting ragged C-termini). tREF and REF could always be detected beside each other, but were not observed on every latex glove sample, which contained Hevein. It was also demonstrated that there is a significant difference in terms of proteins and polymers between inner and outer surfaces of gloves, which helps to explain the different allergenic potential of these.

MALDI imaging allowed for the first time the unambiguous localization of all three allergens in parallel and showed that Hevein was present on 36% of the investigated area of a latex glove with a certain localization, whereupon, tREF and REF were only found on 25% of the investigated material. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: allergy; MALDI imaging; MALDI mass spectrometry; natural latex glove; surface analysis

Introduction

Latex products have become ubiquitously used items in our environment. Biomechanical performance characteristics, like strength, elasticity, tear resistance and superior barrier qualities, made these products essential in many working fields during the last 100 years.^[1] However, in the last three decades, type I allergy against natural latex products became a very serious problem to an increasing number of humans. The prevalence of allergies to latex in the general population is in the range of 2.9–17%.^[2] Up to 64% of patients with urogenital diseases, like spina bifida,^[3,4] and an increasing number of healthcare workers, up to 10%,^[5,6] are affected by this immediate type I allergy (type I) causing lethal anaphylactic shocks in the worst case.^[7–9] Type IV allergies, a delayed contact hypersensitivity localized at the area of contact caused by chemicals as accelerators, antioxidants or secondary preservatives added during the latex production process could be reduced by the introduction of more effective washing steps. Contrary to this T-cell-mediated type of allergy, the compounds responsible for type I allergy are water-soluble proteins,^[10,11] starch-bound proteins^[12–14] and latex-bound proteins^[15] originating from the latex milk, harvested from the rubber tree *Hevea brasiliensis* itself. Whilst water-soluble proteins can easily be removed during production, and cornstarch powder can be avoided by producing powder-free latex products, the latex-bound proteins are physically trapped or strongly adsorbed to the polyisoprene 3D-matrix, and therefore, hard to

remove. Two proteins are regarded as the most crucial allergens in natural rubber products, namely, Rubber Elongation Factor (REF, Hev b1),^[16–21] and Hevein (Hev b 6.02), which is the N-terminal domain of Prohevein (22 kDa).^[8,22–24] REF, a very hydrophobic 14.6 kDa protein, is found to be the major allergen for patients with spina bifida,^[25] whilst Hevein 4.7 kDa is assumed to be responsible for the allergy of healthcare workers to natural rubber products.^[26]

However, patients recognize more than one allergen, and so measurements of total protein content in latex products are now accepted methods for quantitative exposure.^[27,28] Extraction procedures followed by analysis of the extract with a photometric method according to Lowry, or amino acid analysis and immunological assays such as radioallergosorbent tests (RAST) are applied to assess the allergenic potential of surgical gloves. Extraction procedures are vulnerable and easily generate false-positive/-negative protein results.^[29] REF and

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Hevein are quite hydrophobic proteins and it is problematic to extract these proteins quantitatively. Nevertheless, REF and a truncated form of REF (tREF) were characterized recently after a complex extraction procedure from ammoniated latex milk, the alkaline raw material for latex products, by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and electrospray ionization ion trap mass spectrometry (ESI-IT-MS).^[30]

In the past, the extent of different allergenic reactions of individuals to different medical latex gloves was explained by the protein content alone.^[31] How the proteins are presented to the skin of sensitive individuals is not yet understood, and furthermore, it is worth mentioning that so far no correlation has been found between the nature of detected latex allergens, the levels of latex-specific IgE and severity of clinical symptoms.^[32] Owing to the production process^[33] where the latex glove is pulled from a polymerization mould and thereby turned inside out, a difference between the outer and inner surface of the glove has to be considered. In everyday life, healthcare workers are directly in contact with the latter one and show immunoreactions to Hevein and a second allergen, the acidic protein (Hev b 5, 16.1 kDa). This is contrary to patients who are in contact with the outer surface (*Spina bifida* patients) of latex gloves who show reactions to REF, Hevein and a small rubber-particle-associated protein, namely, Hev b 3 (22.3 kDa).^[34]

In the presented study, we tried to overcome the problem of incomplete protein extraction by directly analyzing the surfaces of the latex gloves by MALDI-TOF-MS without any preceding analytical steps. Systematic direct mass spectrometric investigations of inner and outer surfaces of various brands of medical latex gloves are not available up to now. First attempts to address the problem of localizing allergens on the surface of natural latex gloves were already carried out in different, but indirect ways. Peixinho *et al.* showed qualitative and quantitative differences for the inner and outer surface after an extraction procedure followed by immunoblotting using patients' sera^[35] and Grote *et al.* localized the allergens by means of immunogold field emission scanning (FESEM) and transmission electron microscopy (TEM).^[36] A great drawback of both applied strategies is the extraction procedure in the first study and the fact that adequate sera of patients have to be collected for immunoreactions in both cases. Additionally, no structural (e.g. molecular weight) information of the allergens could be determined, and therefore, information on the actual allergy elicitor is missing as cross-reactivity cannot be excluded unambiguously.

MALDI TOF mass spectrometry can determine the presence of allergenic proteins within different brands of latex gloves. The presence or absence of individually known allergens on the inner and outer surfaces can contribute to a better understanding of certain allergic reactions and optimize the production process. Even more important is MALDI MS imaging that allows the construction of two-dimensional distribution maps for REF, tREF and Hevein on a mm-sized piece of latex glove, which can help to get a better understanding of the distribution of these allergens within the top surface layers, and draw conclusions for some still not well understood medical reactions to natural latex gloves.

Materials and Methods

Chemicals

All chemicals and solvents used were of analytical grade except where otherwise stated. MALDI MS matrices, α -cyano-4-hydroxy cinnamic acid (CHCA, ~97%) and 2,5-dihydroxy benzoic acid (DHB, purum $\geq 98\%$), were obtained from Fluka (Buchs, Switzerland) and trifluoro acetic acid (TFA, 'sequanal grade') was purchased from Pierce (Rockford, IL, USA). Formic acid (HCOOH, $\geq 98\%$), acetonitrile (ACN, analytical grade) and water (specific conductance $\leq 1 \mu\text{S}/\text{cm}$) were obtained from Merck (Darmstadt, Germany).

Sample preparation

Nine different brands from various companies manufacturing natural latex gloves were investigated. These sample batches were designated as glove A–H and J. Regarding product information, glove F and H qualified as minimally powdered (starch just on the inner surface) and glove A–E and G are so-called powder-free latex gloves exhibiting no starch powder on the inner surfaces.

The inner and outer surfaces of the gloves (precut into $1 \times 1 \text{ cm}$ pieces) were rinsed with water three times by applying 0.5 ml water on top of the surfaces and immediately blowing it away using a bellow (1 ml pipette tip connected to a Peleus ball) to remove any unwanted residues and further cut into small pieces ($2 \times 2 \text{ mm}$) using a clean scalpel. These pieces were fixed on a stainless steel MALDI target with the side of interest up using a double-sided adhesive Scotch tape (3 M, St. Paul, MN, USA). Before matrix application, the surfaces (already fixed on target) were etched with 0.5 μl TFA and dried at room temperature. 20 mg CHCA dissolved in 1 ml ACN/5% HCOOH (7/3, v/v) and 20 mg DHB dissolved in 1 ml ACN/50% TFA (1/1, v/v) were mixed at a ratio of 1 : 1 (v/v). A volume of 0.5 μl of this freshly prepared matrix mixture was further diluted with 0.5 μl TFA and applied as a droplet directly from the pipette tip on the washed and etched glove surfaces and dried at room temperature.

MALDI mass spectrometry

Mass spectra were acquired on an AXIMA TOF² (Shimadzu Biotech, Manchester, UK) applying a nitrogen laser. The instrument was operated in positive linear ion mode using the molecular mass-optimized delayed-extraction (DE) mode set to m/z 5000 and the accelerating voltage to 20 kV. The ion source pressure during analysis was typically $2.5 \times 10^{-6} \text{ mbar}$ or less. For external calibration, a separate latex glove surface (with the same thickness) was covered with matrix containing calibrants in the interesting mass range, namely, insulin β -chain, cytochrome c and myoglobin. Positive ion mass spectra for inner and outer glove surface analyses were generated by accumulating 500 single, unselected laser shots evenly distributed over 4 mm^2 of the sample. For data evaluation, raw data were baseline subtracted and smoothed using the company-supplied Savitsky-Golay peak processing algorithm.^[37]

MALDI MS imaging analysis was performed by rastering over a final latex glove surface area of $800 \times 400 \mu\text{m}$ in the center of a fixed latex piece. Profiles were acquired by moving the laser over the selected area, whereupon each investigated laser spot was located proximately beside the next one. Each profile represents the result of 200 consecutive, unselected single laser pulses and was stored as raw data, baseline subtracted and smoothed for manual inspection of peak quality. Two-dimensional distribution

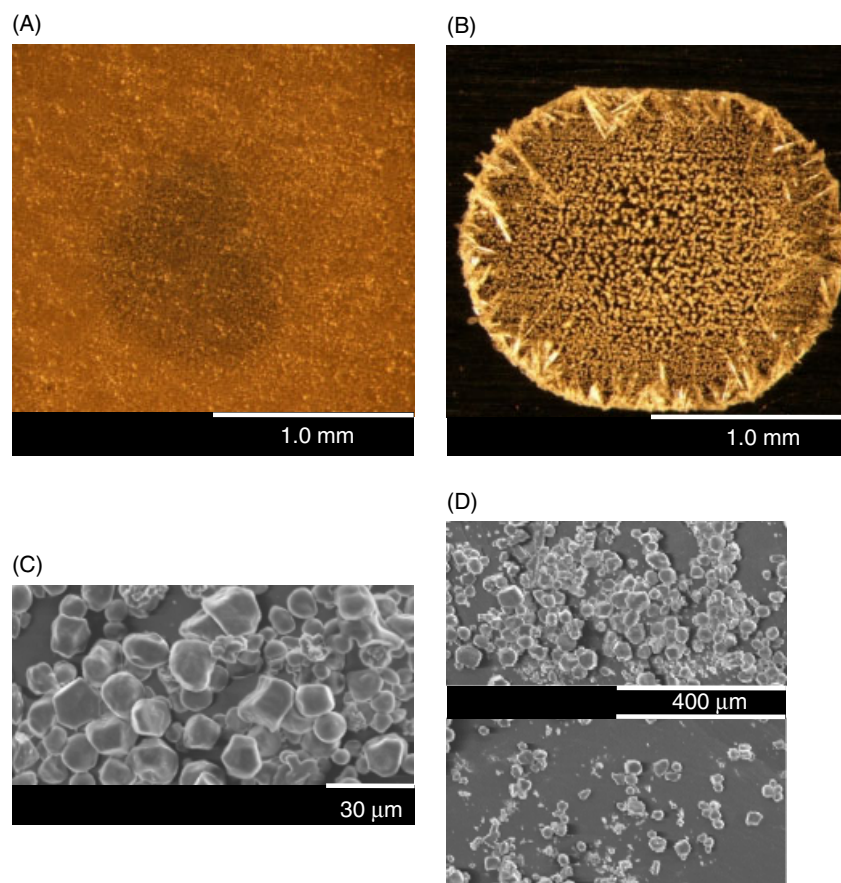


Figure 1. (A) Light microscopy images of a sample preparation of the binary matrix CHCA/DHB deposited on a 2×2 mm latex surface latex glove compared to (B) a conventional dried droplet sample preparation of the same matrix system on a brushed stainless steel target. (C) REM image of binary matrix crystals on the latex surface after drying at room temperature. (D) REM image of an investigated latex surface area of about 800×400 µm before (above) and after (below) MS analysis.

maps of the interesting ions were generated by plotting peak intensities against x–y coordinates of the investigated area. A signal was considered as significant peak when the signal-to-noise ratio (S/N) was better than 3 : 1.

Results and Discussion

Eight different brands of natural latex gloves (glove A–H) were investigated in addition to a reference glove (glove J). Glove J was known not to meet the general criteria^[38] for a natural latex medical glove of low protein content^[39] and immunological innocuousness^[40] because the production process was stopped before the final washing and leaching steps, which would remove proteins and vulcanization additives. Glove H is the finalized product of glove J, after washing and leaching. Gloves A–G were chosen with respect to their label as hypoallergenic gloves and should therefore exhibit low protein contents based on legal standards. Visual observation approved the denotation of the packing of the gloves, where glove D and F qualified as slightly powdered with minor starch residues on their inner surfaces and gloves A–C, E and G as ‘powder-free’ latex gloves, exhibiting no powder on the inner surfaces. As a process-intermediate, glove J was not in contact with any starch and therefore absolutely powder-free as well as its finalized product, glove H.

Sample preparation

To avoid false-positive results for the detection of proteins on the glove surfaces, the powder and other possibly adhesive residues were carefully removed from the inner surfaces. After cutting the gloves into small pieces of about 2×2 mm size, unwanted particles were removed by rinsing the latex pieces several times with water. To guarantee homogeneous sample preparation, all surfaces of all gloves were treated in the same way regardless of whether the glove was powdered or not. After drying at room temperature, the pieces were stuck on the already affixed double-sided adhesive tape on the stainless steel target with the side of interest, inner or outer surface, up. The next step before applying matrix solution, the etching of the surface of interest with TFA, turned out to be a crucial step as no significant signals were detected before this treatment was introduced. This observation might be explained by a leaching process, which mobilizes the proteins by breaking adsorption and similar effects between the latex polymer and the hydrophobic allergens. It was also observed that the TFA roughened the initially smooth surface of the latex. This change of topographical properties of the rubber polymer material possibly gives more degrees of freedom to the proteins for a more efficient desorption/ionization process. Previously described matrix systems well suited for MALDI MS imaging and profiling of proteins and peptides from tissue samples,^[41] as sinapic acid^[42] or ionic matrices,^[43] gave first, but

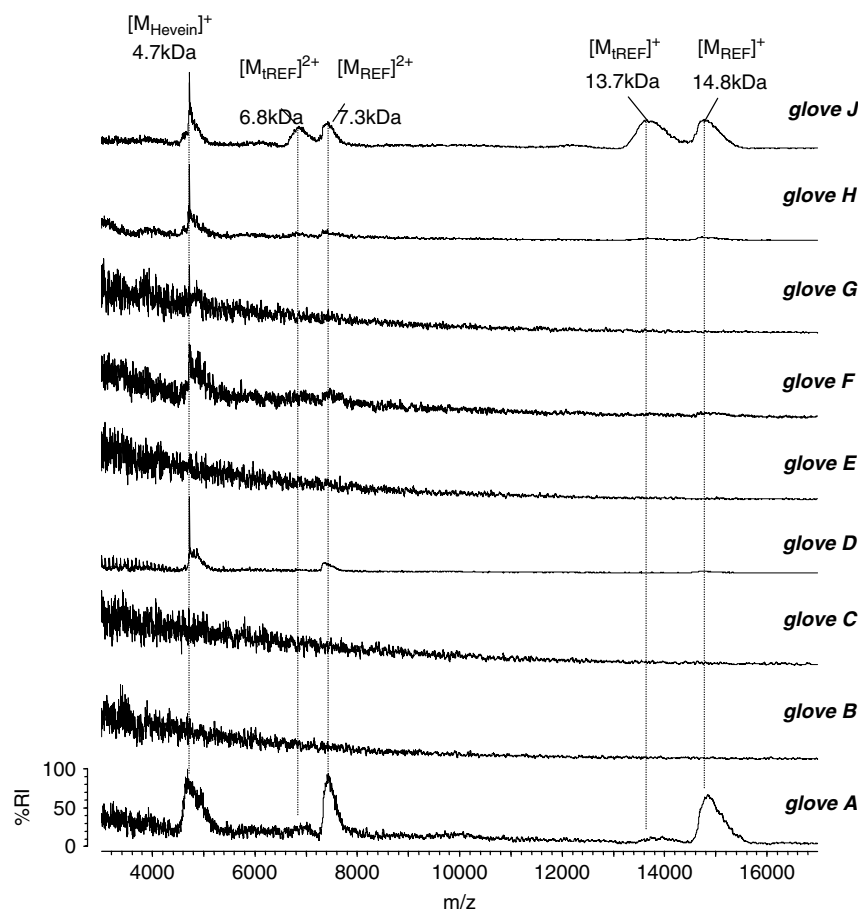


Figure 2. Positive ion MALDI mass spectra in the linear mode obtained directly from the inner surfaces of nine different natural latex glove brands where glove J is considered a reference as it represents an unfinished industrial product (without the final washing and leeching step). Glove H represents the finished product of glove J. (REF, Rubber Elongation Factor; Hev b 1; tREF, truncated REF; Hevein, Hev b 6.02; RI, relative intensity).

very unsatisfying, results in the form of low abundant molecular ion signals with an S/N below 3. Beside the low signal intensities, only 'sweet spot analysis' was possible where analytes could be desorbed and ionized only from one or maybe two single positions on the latex surface although the matrix deposition was very homogenous for these preparations. These findings were useless for the subsequent MALDI imaging experiments. A binary matrix system consisting of CHCA and DHB was adapted for the presented surface analysis. One of the most important reasons for choosing this matrix composition was the fact that this system has been described as to be very tolerant for salt contaminants.^[44] It was therefore considered to reduce adduct formation arising from still present salts, which could not be removed by diligent and multiple washing steps before fixing the samples on the MALDI target. Additionally, published data described DHB as an efficient matrix for hydrophobic compounds,^[45–47] which is optimal for proteins like REF. The addition of CHCA should overcome the drawback of unevenly distributed matrix crystals across the investigated sample area, which has been observed for DHB in many studies. This uneven distribution leads to preferential desorption/ionization of analytes across the inspected area,^[48,49] which has to be prevented for subsequent imaging experiments. To obtain better reproducibility and adequate S/N, the formation of small matrix crystals is sought.^[50] As shown in Fig. 1(A), evenly distributed matrix crystals were observed on the surfaces of the latex gloves using the binary matrix system CHCA/DHB. The matrix

crystals on the latex surfaces were small (about 15 μm), almost round, and spread over the sample with only very tiny uncovered regions (Fig. 1(C)). This is contrary to crystallization behavior on a brushed stainless steel target where cubic crystals were observed in the center of the spot, typical for CHCA, and longish ones (typical for DHB) at the outer rim (Fig. 1(B)). It can be concluded that the rough morphology of the latex, additionally provoked by the etching step in the beginning, is favoring the formation of the preferred crystal form. Compared to the diameter of the laser beam (approx. 30–50 μm), occurring uncovered surface areas between some of the crystals, as it can be observed in Fig. 1(C) and (D), could be neglected. Matrix was evenly removed over the investigated area as it can be observed from the desorbed, but still partly present, matrix crystals after laser irradiation (Fig. 1(D bottom)). Best results for the analyses of latex surfaces were obtained for a solvent system finally containing 30 vol% organic and 56.6 vol% acidic modifier in an aqueous solution (13.3 vol%). This is in contrast to biological tissue MS imaging where the organic/aqueous ratio was suggested to be 1/1 for optimal results.^[42] To overcome the previously described phenomenon of varying ionization efficiencies for different analytes under different pH conditions^[51] and differing responses for peptides up to 11 kDa using solvents of differing pH,^[52] a combination of two different acidic modifiers, HCOOH 0.38 vol% and TFA 56.3 vol%, was chosen.

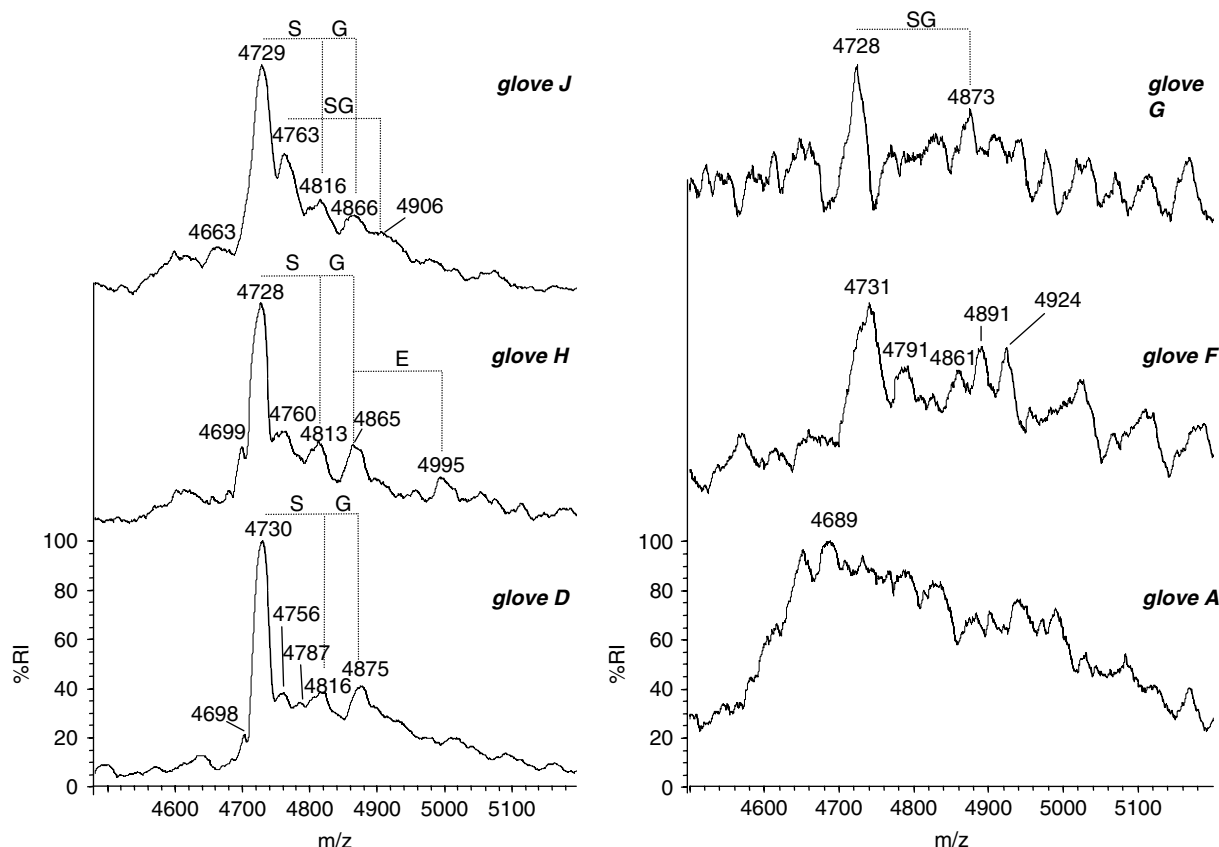


Figure 3. Detailed view of the m/z range related to the molecular ion region of Hevein of the MALDI linear TOF mass spectra generated from the inner surfaces of six natural latex gloves. For glove D, G, H and J the tentative ragged C-termini have been assigned with possible amino acid sequence extensions.

MALDI mass spectrometry of the inner and outer surface of medical latex gloves

As can be seen in Fig. 2, all three major allergens, REF, tREF and Hevein were observed in the positive ion MALDI mass spectra on the inner glove surfaces applying the described optimized sample preparation protocol. Hevein (theoretical average M_r , 4728 Da) was detected as a distinct peak (protonated molecule) at an average molecular mass of 4729 ± 4 Da for glove D, H and J (Fig. 3). For these gloves, major peaks at m/z 4816 and 4866 were additionally detected with the same precision. These peaks could not be explained simply by adducts (e.g. alkali or matrix) but gave evidence for protein modifications. The fact that Hevein has a ragged C-terminal sequence with extensions, namely, the addition of Ser and of the dipeptide Ser-Gly, has already been described^[53] and two of these peaks, m/z 4816 and 4866, could be explained by this modification within the precision achieved for these experiments. The third peak (m/z 4995) has not been described yet and was occurring as a significant peak only in the MALDI mass spectrum of glove H. The mass difference can be explained by an additional C-terminal Glu, giving a C-terminal Ser-Gly-Glu sequence, which is in agreement with the published sequence for the Hevein precursor (HEVE_HEVBR P02877). The other signals for gloves J, H and D could not be explained by published Hevein sequence data, but it is noticeable that the mass difference between m/z 4763 and 4906 for glove J again indicated a Ser-Gly sequence. The very small peak at m/z 4699 for glove H and m/z 4698 for glove D was in good agreement with published sequence data for Minor Hevein (HEVP_HEVBR P80359, theoretical

average M_r , 4695 Da), a Hevein homolog where seven amino acids are exchanged, indicating a co-existence of these two Hevein isoforms.

Gloves A, F and G did not give results as clearcut as the data obtained for J, H and D. For glove G, only a very small peak with an $S/N < 3$ was detected for Hevein at m/z 4728. An additional peak at m/z 4873, $S/N < 3$, might again be associated with an additional Gly-Ser residue. For glove F, an average molecular mass of 4731 ± 4 Da was observed for Hevein. Small additional peaks observed in gloves G and F, not detected in the previously discussed brands (gloves D, H, J), could not be explained by described Hevein modifications. For glove A, only a very broad peak with a peak maximum at m/z 4689 was detected and it is assumed that this peak also corresponds to Hevein and modified forms of this protein. This very unfavorable peak shape can be explained by various effects. First of all, alkali adducts as well as metastable decay could not be unambiguously excluded during these studies leading to asymmetric peak-tailing effects despite a washing procedure. Additionally, the desorption/ionization process of proteins tightly associated with certain surfaces/materials, namely, the polyisopren 3D structure (similar to 3D structure of polyacrylamide), might be a more difficult process leading to an expanded spatial distribution of ions formed in the source. The nonuniform topology of the latex material additionally contributes to unequal analyte desorption in terms of time and space affecting peak width, which can no longer be corrected by time-delayed extraction. Trying to enhance the desorption process by reducing possible surface-associated charging effects, which also negatively influence the flight time of

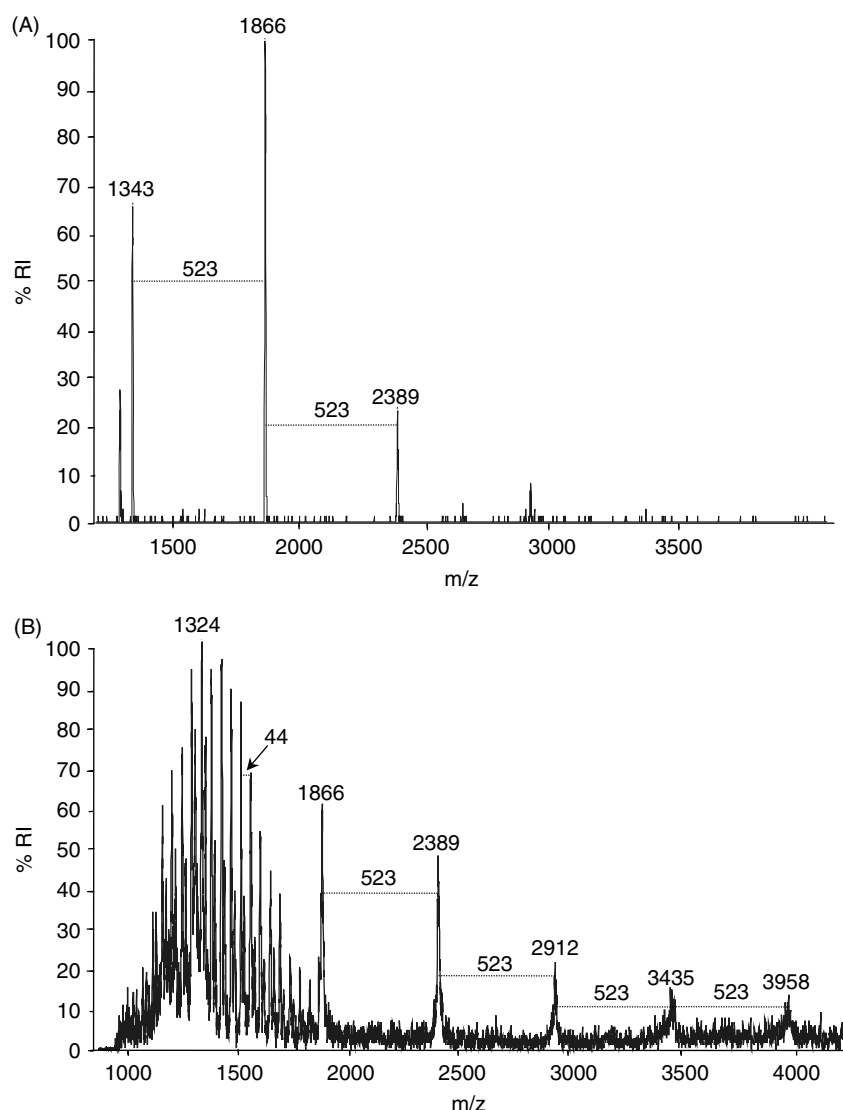


Figure 4. The m/z 1000–4000 range of the MALDI linear TOF mass spectrum of glove B. (A) Inner surface, and (B) outer glove surface.

ions in the timed-ion regime^[54] by coating the samples with gold using a sputter coater did not improve the results. On the contrary, no proteins of interest could be detected.

Single-charged molecules of REF (theoretical average M_r 14.6 kDa) and tREF (theoretical average M_r 13.6 kDa), as well as their doubly charged molecules, were detected on gloves A, D, F, H and J (Fig. 2). All these peaks had a very broad peak shape, which might be explained by the same reasons discussed above. Interestingly, glove A exhibited a very intensive signal for REF at m/z 14859 (± 71 Da), which could be compared to the signal intensity of Hevein, but only a very low intensity signal at m/z 13747 (± 89 Da) with $S/N < 3$ corresponding to tREF was detected. Glove D exhibited a signal only for REF at m/z 14804 (± 68 Da) at $S/N \approx 2$ for the singly charged molecule, but a distinct signal ($S/N \approx 4$) was observed for the doubly charged molecule ($[M_{REF}]^{2+}$ m/z 7356 Da). For glove F, the MALDI mass spectrum exhibited only a very faint signal for REF, and also the presence of the doubly charged molecule of REF and tREF was of very low intensity ($S/N < 3$). But a hint for the presence of both allergy elicitors is given. Glove G was the only glove where a signal for

Hevein could be detected, but clearly, no signals for REF or tREF were observed. As glove H represents the final product derived from glove J, the comparison of the MALDI mass spectra of these two inner surfaces emphasized the necessity of the final washing and leaching steps during manufacturing. In both gloves, REF and tREF were detected, but signal intensities were many times smaller for glove H (just residues were detectable). The peak intensity for REF [glove H: m/z 14712 (± 81 Da); glove J: m/z 14779 (± 78 Da)] dropped below 8% relative intensity (RI), tREF [glove H: m/z 13689 (± 45 Da); glove J: m/z 13653 (± 51 Da)] below 6% RI and Hevein (glove H: m/z 4728 (± 4 Da), glove J: m/z 4729 (± 4 Da)] below 50% RI for glove H compared to the signal intensities obtained from the same proteins in glove J (the reference glove) giving, therefore, indications for the effectiveness of the finishing steps in production. As the signal of Hevein was already very weak for glove G no signals for REF and tREF could be observed.

For the inner surfaces of glove B, C and E and the outer surfaces of all analyzed brands, no signals for the allergens were obtained. However, as can be seen in Fig. 4, for glove B interesting signal distributions between m/z 1000 and 4000 were observed on the

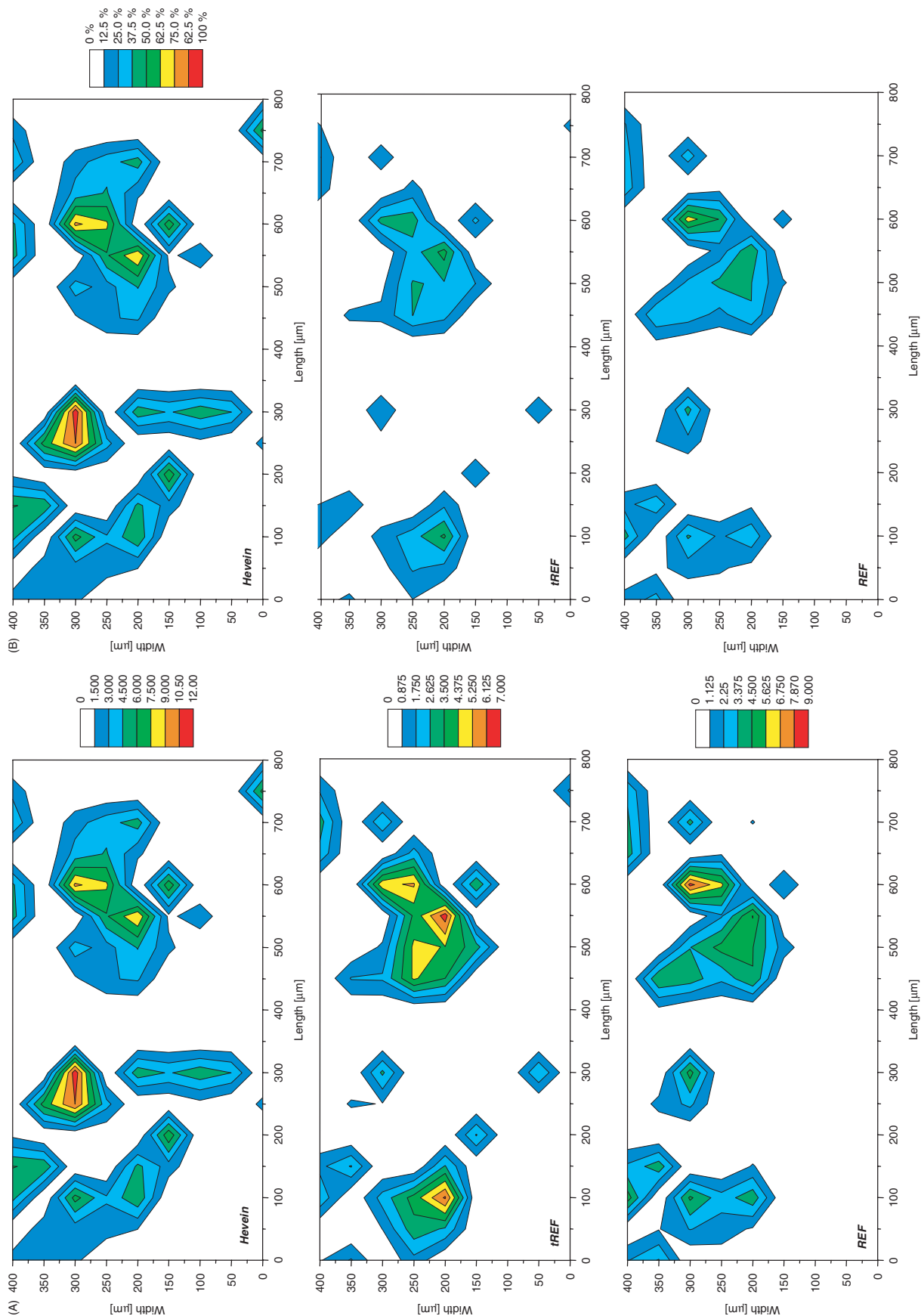


Figure 5. Intensity plots for the distribution of Hevein, tREF and REF on a 400 × 800 μm piece of latex glove J. (A) The contour plots represent absolute intensity distributions for each allergen from the lowest (0 mV) to the highest intensity (12 mV Hevein, 7 mV tREF, 9 mV REF). (B) The contour plots represent relative intensity distributions. tREF and REF were referred to the most intensive signal of Hevein (100% equals 12 mV).

inner and outer surfaces. The signals at m/z 1343, 1866 and 2389 with mass differences of 523 Da were detected on both surfaces, and the signals at m/z 2912, 3435 and 3958 additionally observed on the outer surfaces could be associated with the same polymer distribution. These distributions may result from lubricants, often polymers, added during the production process to smoothen the surfaces. On the outer surface area, this polymer distribution was overlaid by a second one between m/z 1000 and 1800. A mass increment of 44 Da allowed the latter one to be assigned to a polyethylene glycol (PEG). In fact, PEG is a well-known antifriction coating added during the last steps of the production process to smoothen the gloves' inner surfaces.

It has to be mentioned that it was not possible to prove the identity of Minor Hevein, tREF and REF by mass spectrometry. The troublesome desorption/ionization behavior of the latex-particle-associated proteins completely prevented effective MS/MS experiments. The intensity of the precursor ion was not sufficient enough throughout the experiments to generate sequence data. Enzymatic digestion carried out directly on the surface, which has been shown to be applicable for protein identification in biological tissues,^[55] was also not successful as only one peptide above 800 Da can be expected for Minor Hevein (calculated from the theoretical sequence), and this peptide ($[M + H]^+$ theor. m/z 2730) could not be clearly observed as the signal overlapped with signals from other analytes present in the latex surface (e.g. lubricants, polymers). Although more than one peptide larger than m/z 1000 was expected for tREF and REF (7 and 8 peptides respectively), no specific signal was observed because of the same reasons discussed for Minor Hevein. Therefore, the presence of the allergens was confirmed using specific, specially produced, polyclonal antibodies for Western blotting of the latex glove surfaces (data not shown). These experiments were relevant for the presence of the allergens on the surfaces. The overall presence was also confirmed after protein extraction^[30] and separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (data not shown).

MALDI mass spectrometric imaging

The inner surface of reference glove J was chosen to evaluate the imaging potential for constructing protein distributions on an $800 \times 400 \mu\text{m}$ piece of latex glove due to the fact that on this glove sample intensive ion signals for all three allergy elicitors were obtainable (Fig. 2). The peak maxima of the signals within the determined precision for Hevein ($[M + H]^+$ m/z 4729 ± 4), REF ($[M + H]^+$ m/z 14.8 ± 0.1 k, $[M + 2H]^{2+}$ m/z 7.38 ± 0.07 k) and tREF ($[M + H]^+$ m/z 13.70 ± 0.09 k, $[M + 2H]^{2+}$ m/z 6.83 ± 0.05 k) were used for analysis if the S/N was at least exceeding 3. As shown in Fig. 5(A), the most intensive signals for Hevein are localized in certain areas and no equal distribution is found across the scanned area. REF and tREF were, to a certain degree, colocalized, and again no uniform distribution across the whole area was found. Interestingly, Hevein was observed on areas where dominating signals for tREF and/or REF could be detected. This fact speaks in favor of a uniform desorption/ionization process for all three allergens without any significant ion suppression effects, which might have biased the imaging results. Interestingly, the distribution of tREF was always accompanied by the presence of REF, although the absolute intensity for the most abundant tREF signal (7 mV) was lower than the most intensive signal for REF (9 mV), which again was less intensive than Hevein (12 mV). For a better understanding of relative peak intensities,

these were scaled to the most intensive peak (12 mV) of Hevein (Fig. 5(B)). It was observed that Hevein could be detected on about 36% of the totally investigated area, but only 25% for tREF and REF with an S/N > 3. MALDI mass spectrometric imaging applying the latex optimized sample preparation allowed the unambiguous detection and localization of all allergens of interest in parallel without the uncertainties of immunoanalytical methods, which makes this approach outstanding compared to previously published data.^[35,36]

Conclusion

In the presented work, we could demonstrate that it is possible to detect the major allergy elicitors directly from the inner and outer surfaces of commercially available natural latex gloves. This approach allows a fast assessment of the allergenic potential of gloves with respect to the presence of the interesting allergens without any preceding protein extraction (e.g. cutting the latex gloves into tiny pieces and extracting the proteins with suitable surfactant solutions) or availability of adequate sera for immunological results. One essential step for the successful desorption/ionization by vacuum MALDI of the tightly bound allergens REF, tREF and Hevein was the sample preparation. Etching the surfaces with TFA seemed to mobilize proteins from deeper regions of the latex material and/or reduces the latex (polyisopren)-protein interaction. The surface modification process may allow the swelling of the narrow latex 3D structure, and therefore, loosening the cage-like structure. This may favor an easier desorption/ionization process. Under these sample preparation conditions, it could be shown that Hevein, REF and tREF can be desorbed and ionized directly from the latex material giving information on the exact molecular mass of present proteins (and in some cases of added polymers) and relative quantities within one brand. MALDI mass spectrometry in the linear mode allowed the comparison of various latex glove brands where different modifications of Hevein were detected, which could partly be assigned to already described C-terminal variations. It was observed that four different forms of Hevein (Hevein, Hevein-Ser, Hevein-Ser-Gly, Hevein-Ser-Gly-Glu) were present within one latex product, three of these forms were present in another product and one or two forms were present in two other brands. tREF and REF showed diverse behavior in terms of presence/absence and relative intensities in the different glove brands. In five out of nine latex glove brands, these allergens were present, beside Hevein. In two gloves, these proteins were not detected although Hevein was present, and in two other gloves, Hevein, REF or tREF were not detected. On the outer surfaces, only polymers with different distributions could be detected, but not allergens.

The first MALDI imaging method for a natural polymer (latex) surface was developed and the spatial distribution of the three most important latex protein allergens was shown. This turned out to be a fast and easy strategy to analyze in parallel various allergens without the difficulties of immunoanalytical methods. Furthermore, this is the base for the development of a future MALDI profiling method.

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