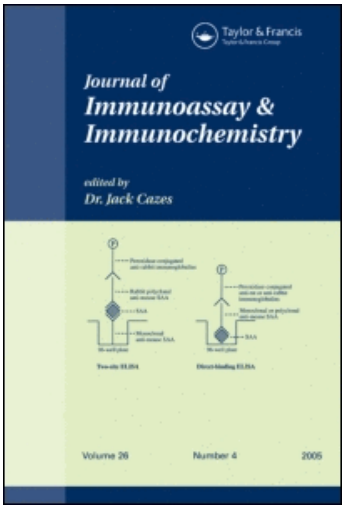

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Binding and Measuring Natural Rubber Latex Proteins on Glove Powder

Vesna J. Tomazic-Jezic[a]; Anne D. Lucas[a]; Beatriz A. Sanchez[a]
[a] Center for Devices and Radiological Health, Division of Life Sciences, USFDA, Rockville, Maryland, USA

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Binding and Measuring Natural Rubber Latex Proteins on Glove Powder

Vesna J. Tomazic-Jezic,* Anne D. Lucas, and Beatriz A. Sanchez

USFDA, Center for Devices and Radiological Health, Division of Life Sciences, Rockville, Maryland, USA

ABSTRACT

Cornstarch used as a donning powder on natural rubber latex (NRL) gloves adsorbs NRL proteins. During glove use, powder-carried proteins can be aerosolized and can cause allergic reactions in NRL sensitized individuals. The amount of NRL proteins bound to glove powder and its relative relationship to the total amount of proteins on the glove has not been studied, due to the difficulty in measuring proteins on powder. Using the ELISA inhibition assay for NRL proteins [Standard test method for the immunological measurement of antigenic protein in natural rubber and its products. In: *The Annual Book of ASTM Standards*; ASTM: West Conshohocken, PA, 2000; ASTM D 64-0] we have investigated possible protocol modifications in order to include measurement of proteins bound

*Correspondence: Vesna J. Tomazic-Jezic, USFDA, Center for Devices and Radiological Health, Division of Life Sciences, HFZ-112, 12709 Twinbrook Parkway, Rockville, MD 20852, USA; Fax: (301) 596-6775.

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to glove powder, as well as the water-extractable glove proteins. Possible interference of the starch itself was evaluated by adding clean cornstarch to the assay. No significant interference was observed with powder concentrations below 5 mg/mL. We analyzed 19 extracts of powdered surgical and examination gloves before and after removal of the particulate component. Comparison of NRL glove extracts with, and without, the cornstarch powder fraction indicated significant variations in the ratios of powder-bound protein and corresponding water-extractable protein. The ratios did not appear to correlate with either the total protein on the glove, the glove weight, or the total amount of powder on the glove. However, when virgin glove powders were exposed to NRL proteins, binding was proportional to the protein concentration in the suspension. Temperature in the range from 4°C to 37°C, did not affect binding intensity, while a higher pH resulted in a higher level of protein associated with, or bound to, the starch. The major differences in the propensity for NRL protein binding were observed among different glove powders.

The data indicate that the amount of protein that binds to glove powder does not depend only on the initial protein levels in the raw NRL. More likely, other physical or chemical factors introduced during the manufacturing process, as well as the properties of the donning powder itself, may influence protein binding. Moreover, we demonstrated that the ELISA inhibition assay could be successfully modified for quantitation of proteins adsorbed on the glove powder, together with water-extractable proteins.

Key Words: Natural rubber latex; Latex proteins; Glove powder; Immunoassay; Latex allergy

INTRODUCTION

The use of powdered natural rubber latex (NRL) gloves is linked to a variety of allergic reactions in sensitive people[1–7] and is implicated in other health problems (reviewed in[8,9]). Cross-linked cornstarch, most commonly used as a glove donning powder, adsorbs NRL proteins,[10,11] which becomes airborne during glove use,[12,13] creating an allergenic aerosol.[14–16] The inhaled aerosolized allergens come in direct contact with mucous membranes and are considered a major cause of respiratory problems in sensitized individuals. Because the mucous membranes allow more absorption than the intact skin, inhalation may present a significant route of sensitization for both the glove users and the non-users in the proximity of the user area.

The main approach to minimizing the allergenic potential of NRL products, has been focused on reduction of the protein amount on finished products. The American Society for Testing and Materials (ASTM) developed two standard methods for the assessment of the extractable protein concentration of NRL products: the Modified Lowry assay D5712,[17] which

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measures total protein content, and the ELISA inhibition assay D6499,[18] which measures antigenic NRL proteins. Both of these methods are considered indicators of potential allergenicity, but they measure only the water extractable fraction of total NRL proteins. Proteins bound to the donning powder have not been included in the measurement by these or any other commonly used methods. Because the proportion of the powder-bound protein vs. water-extractable protein for the NRL products is not known, the total level of protein on powdered gloves cannot be extrapolated at this time. Attempts have been made to quantitate protein in the airborne starch released from powdered gloves and in the starch slurries during the manufacturing process.[19–22] These studies indicate that the amount of powder-bound protein depends not only on the protein level in raw source material, but also other factors may affect protein binding to glove powder during manufacturing procedures.[19,21] Therefore, one could assume that the levels of protein on the airborne glove powders may not necessarily be proportional to the levels detected in the water extracts of NRL gloves.

It is important to note that studies addressing the levels of airborne allergen refer only to the amount of protein or allergen that is extractable from the dry airborne glove powder, or the powder in manufacturer's slurry tanks. The portion of protein remaining on the powder after extraction has not been included in measurements, because none of the presently available methods for quantitation of NRL proteins can measure powder-bound proteins.

One of the aims of this study was to modify the ELISA inhibition assay (the ASTM D6499) to include measurement of the powder-bound protein. With the modified procedure, we evaluated the differences in protein levels in glove extracts with, and without, respective powder fractions. Furthermore, several virgin glove powders were evaluated for their capacity and/or affinity to bind NRL proteins under various physical–chemical conditions.

EXPERIMENTAL

Reagents

The preparation of NRL protein extract from ammoniated latex (AL) and non-ammoniated raw natural rubber latex (NAL) has been described earlier.[23] The protein sources used in this study were: NAL, prepared in our laboratory; FDA reference NAL protein E-8; and AL, the ASTM standard antigen prepared for the D6499 protein assays. The virgin glove donning powders included: two cross-linked cornstarch glove powder preparations (starch #1 and #2); commercially available household cooking cornstarch (starch #3); and an oat starch glove powder (#4).

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Protein Extraction from NRL Gloves

Gloves evaluated in this study included presently marketed, powdered, surgical, and examination NRL gloves from a variety of manufacturers. Proteins were extracted according to the standard ASTM protocol.[17] Briefly, extraction was conducted in 0.1 M PBS, pH 7.4 for 2 hr at 37°C temperature with shaking. Immediately after extraction, the solid pieces of gloves were removed. One half of each glove extract containing glove powder was saved immediately; it will be referred to as the "whole extract." The other half of each glove extract was centrifuged to remove all particulate material and referred to as the "clear extract."

Exposure of Virgin Starch to NRL Proteins

The starch samples were exposed to NRL proteins to evaluate the significance of starch type, protein concentration, temperature, and pH on their binding propensity. Dry, clean samples of each starch preparation (100 mg) were aliquoted into polypropylene tubes, washed with either water or PBS and exposed overnight to 1 mL solution of NAL or AL proteins of various concentrations. The exposure was either in water or PBS at the pH 5.7, 7.4, and 9.0, with three temperatures ranging from 4°C to 37°C. After incubation, starch suspensions were centrifuged and supernatants removed and saved. Starch pellets were washed and re-suspended in the PBS, pH 7.4.

Protein Assay Protocol

The ASTM standard ELISA inhibition assay (D6499) was used to measure protein levels in whole and clear glove extracts, and also in the exposed starch samples and corresponding supernatants. For the assay of the samples containing cornstarch powder, the standard protocol was slightly modified. The inhibition step was performed with continuous shaking at room temperature. After 2 hr inhibition step, the starch-containing plates were centrifuged and the clear supernatant was transferred into the assay plates. The remaining procedure was identical for all samples, as described in the ASTM standard protocol.[18] Because the ELISA inhibition is a two step assay, starch was present only in the inhibition step, and did not cause any interference in the final step of the assay. The possible interference of cornstarch donning powder alone on the ELISA assay was evaluated by making serial dilutions of standard NRL protein, and adding various amounts of virgin glove powder. The standard curves generated by the NRL proteins

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with different amounts of powder were compared to standard curves of NRL proteins in buffer alone.

Statistical Evaluation

Most of the values presented here are calculated as a mean of duplicate samples from two independent experiments. Standard deviations were not higher than 10%. A student *t*-test was performed to determine statistically significant differences. The data groups with $p > 0.01$ are labeled.

RESULTS

The evaluation of possible interference of powder alone in the ELISA inhibition assay showed no effect on the performance of the test at or below 1 mg/mL (Fig. 1), and only minimal impact on the assay was seen at 5 mg/mL and higher. With adoption of the ASTM proposed limits, most of the gloves would have powder levels below that amount, indicating that the

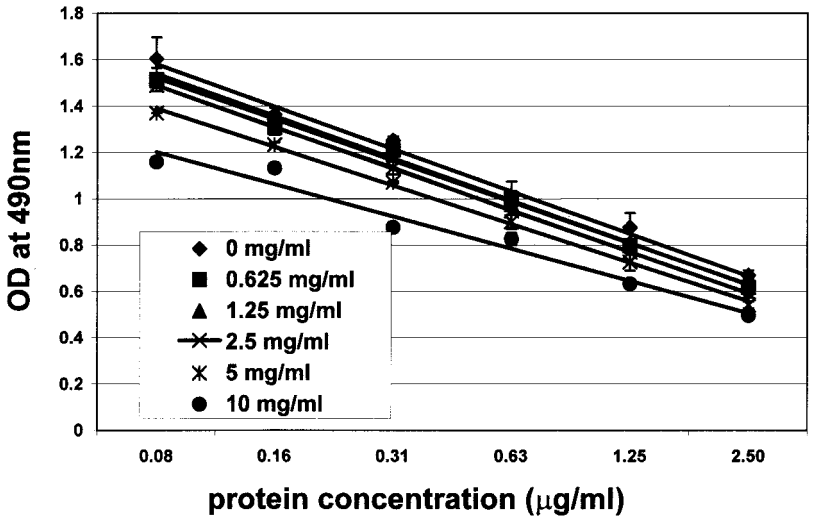

Figure 1. The effects of various concentrations of donning powder (0–10 mg) on the performance of the ELISA inhibition assay. A clean cornstarch powder was added to a series of standard antigen dilutions. Values represent means of duplicate samples from two separate experiments.

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ELISA inhibition assay can be satisfactorily used for the evaluation of protein levels in glove extracts without removal of glove dusting powder.

Applying the modified D6499 assay, we evaluated protein levels in the clear and the whole extracts of 18 randomly selected powdered, surgical and examination NRL gloves. The results showed higher protein levels in all whole glove extracts in comparison with the respective clear extracts (Fig. 2). However, the ratios between protein levels in whole and clear extracts were not uniform. The whole extracts had protein values in the range of 12–25% higher than the corresponding clear extracts, with a few samples even higher.

To investigate the possible reasons for this disparity between the protein levels in the clear glove extracts and the corresponding powder-containing whole extracts, a series of experiments was performed to evaluate factors that may increase or decrease the amount of NRL proteins adsorbed on glove powder. A cornstarch sample (starch #2) was exposed overnight to

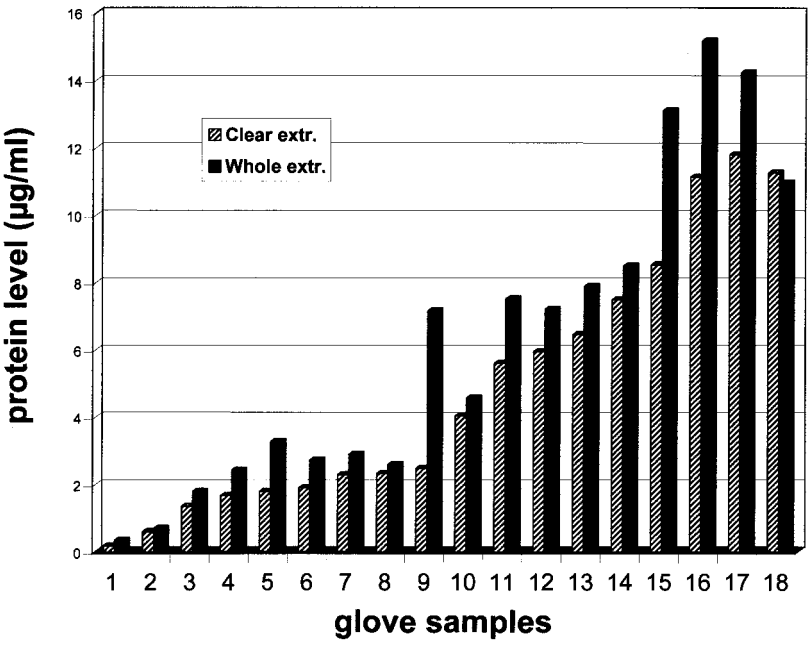

Figure 2. Analysis of the protein levels of powdered NRL gloves. The whole extracts containing glove powder were compared to respective clear extracts, where powder fraction was removed. The values represent the mean of duplicate samples from two independent experiments. SDs were less than 10%.

various concentrations of NRL protein at 4°C, at room temperature and at 37°C, with occasional shaking. The data indicated that the temperatures in the range of 4°C to 37°C had no effect of on the amount of protein binding (Fig. 3). However, the amount of protein bound to the starch was dependent of the starting concentration of protein in the exposure solution. The amount of protein bound to starch increased with increasing protein concentration in the solution. From a background level of approximately 2 μg/100 mg of starch, under the conditions of our study, about 8–10% of the protein in solution bound to the virgin cross-linked cornstarch. An apparent saturation was reached when starch was exposed to 200 μg/mL of protein.

Considering that NRL proteins comprise a large number of individual proteins with various charges and isoelectric points, the significance of pH in protein binding to starch was evaluated. The amount of bound protein at various pH was evaluated for two cross-linked cornstarch preparations and one preparation of oat starch that was not cross-linked. There was no difference in the level of protein binding at pH 5.7 and 7.4, but somewhat higher binding was observed at pH 9.0. The difference in protein binding of three

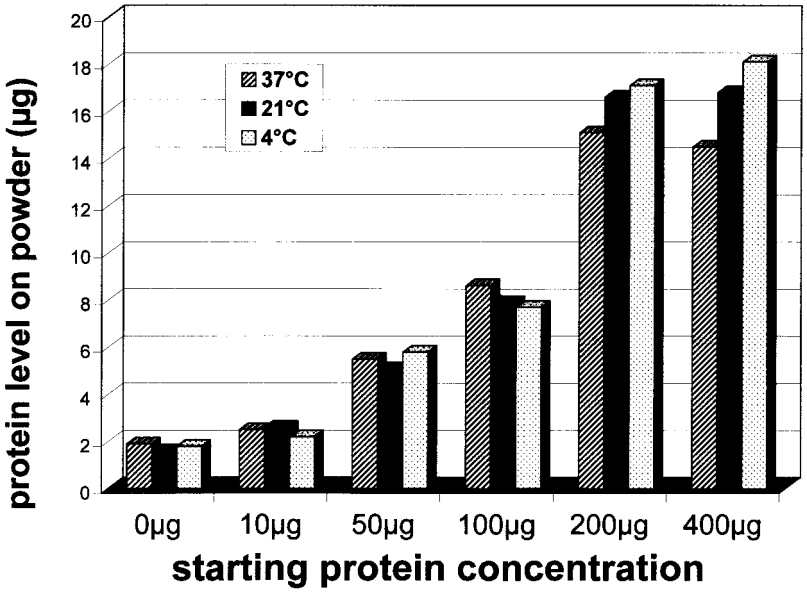

Figure 3. The effects of temperature and NRL protein concentration on the amount of protein binding to powder. A cross-linked cornstarch (#2) was used in this study. Starch was exposed to NAL proteins in PBS, pH 7.4. The values represent the mean of duplicate samples from two independent experiments. SDs were less than 10%.

starches exposed to buffers at pH 7.4 and pH 9.0 is shown in Fig. 4. The two cross-linked cornstarches (#1 and #2) bound more NRL protein at the pH 9.0 than at the pH 7.4. We observed no significant pH effect on the amount of protein bound to the oat starch (starch #4), but the total amount of protein measured on this starch sample was much lower than on the two other starches.

In the manufacturing plants there is no pH control and all processing is conducted in tap water. To create similar conditions, we performed the experiment with starches exposed to protein suspended in water instead of PBS. In addition to three starches studied earlier, we added to this experiment a commercially available cooking cornstarch (starch #3). All starches were exposed to 400 μg of either AL or NAL proteins. The protein level was measured in

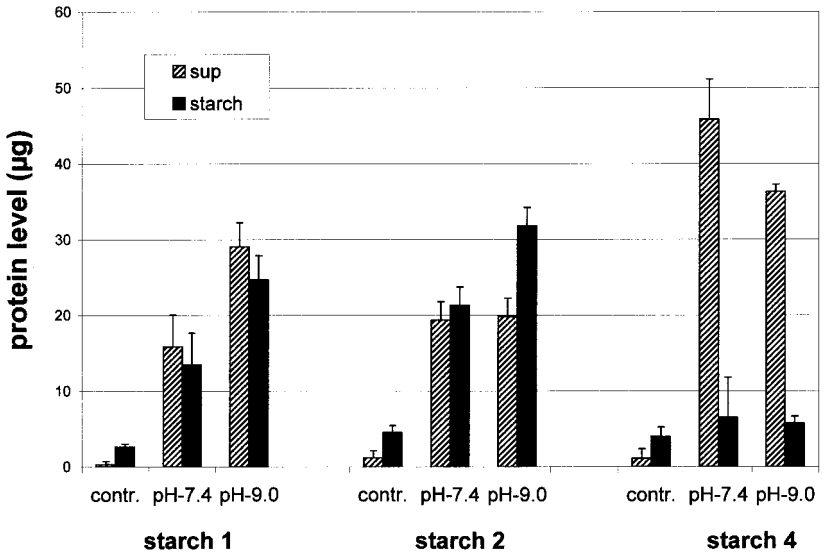

Figure 4. Comparison of the NRL protein binding capacity of three glove powder samples. Starch 1 and 2 are cross-linked cornstarch powders, starch 4 is an oat starch dusting powder. Powder samples (100 mg) were exposed to 400 μg/mL NRL proteins in buffers at pH 5.7, 7.4, and 9.0. Data for pH 5.7 samples are not shown as they were the same as values for pH 7.4. The values for the starch reflect the total amount of protein on 100 mg of starch. Supernatants (sup) values are 1/10 of the total protein recovered. Each value is a mean of duplicate values from two separate experiments. The controls represent baseline values for each starch sample without protein added. There was no difference in control values at different pHs. The values represent the mean of duplicate samples from three independent experiments. SDs were less than 10%.

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recovered supernatant and the respective washed starch pellet. We did not observe a marked difference in binding patterns of AL and NAL proteins, except for starch 1, when exposed to proteins in PBS (Fig. 5). Starches #1 and #2 bound substantially more protein in water than in PBS, especially starch #1. Both starches also bound substantially more protein than starches #3 and #4. Starch #3 appeared very similar to starch #4 in binding capacity. Because of the low levels of protein bound to starches #3 and #4 relative to starches #1 and #2, the slightly higher binding in water compared to PBS was not statistically significant ($p < 0.01$). The important finding here is a significant difference in the propensity of starches #1 and #2 to bind NRL proteins, in comparison to starches #3 and #4, especially when exposed to NRL proteins in water ($p > 0.01$). Furthermore, the calculated total amount of protein measured on powders #3 and #4, and the total amount of protein recovered in the respective supernatants, was close to 100% of the starting amount of protein. On the other hand, in the case of starches #1 and #2, the amounts of protein recovered on starches and in respective supernatants were between 30% and 60% of the total amount (Fig. 6). It seems, that in the last two cases a substantial amount of protein has been lost by washing starches after the supernatants were removed. This finding indicates that on starches #1 and #2, a portion of protein may have been only loosely bound, or adsorbed on the surface, and was released during the washing procedure.

DISCUSSION

The ASTM ELISA inhibition assay (D6499), designed to measure extractable antigenic NRL proteins, can also be applied to measure the amount of proteins associated with the glove powder. With minor modifications described in the Methods, the whole glove extract without the elimination of powder can be used in the assay. When glove extraction is performed according to the ASTM standard, the powder level in most of the extracts would be below 5 mg/mL, which is the level under which no marked powder interference was observed. We measured the extractable protein levels on NRL gloves, comparing the whole extracts with the corresponding clear extracts for each of 18 gloves. The protein levels in whole extracts were consistently higher than those in corresponding clear extracts, indicating that some proteins remain bound to the glove powder after the extraction. The levels of powder-bound protein, however, were not proportional to the protein levels in the clear extracts. In the whole extracts, protein levels were mostly in the range of 8–25% higher than in the corresponding clear extracts; with a few gloves the difference was even higher. This finding indicates that protein binding to glove powder is affected by other factors, in addition to the starting

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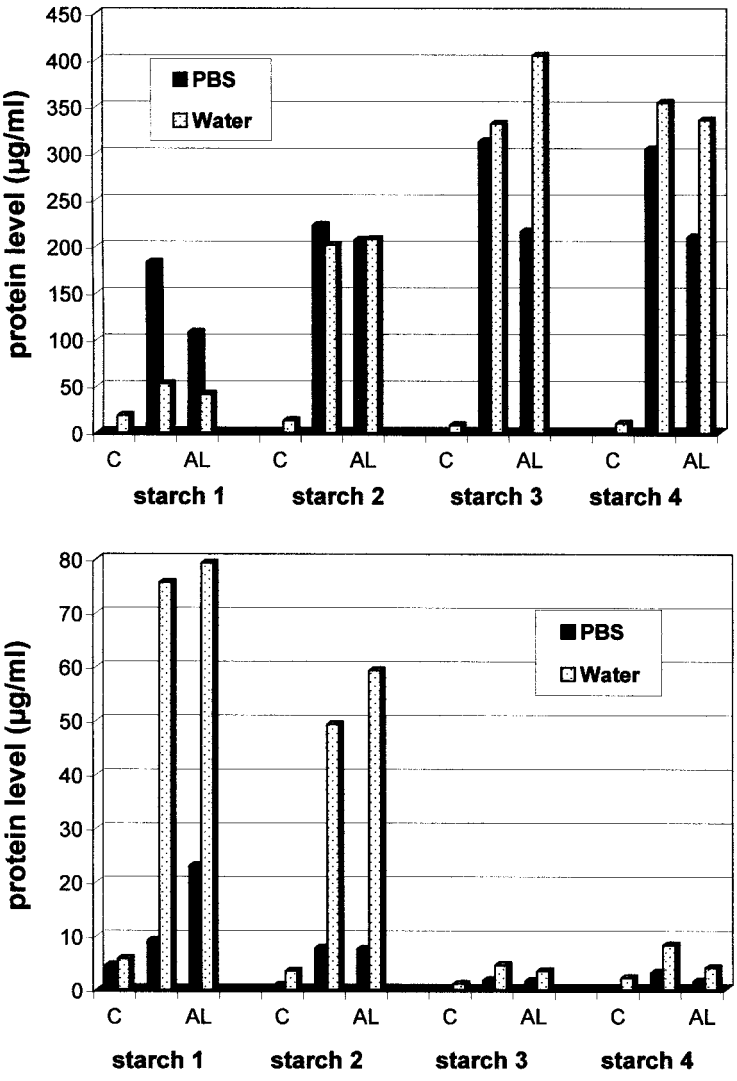

Figure 5. Comparison of protein binding to starches exposed to NRL proteins in water and PBS, pH 7.4. Starch samples (100 mg) exposed to 400 µg of NRL protein. A total amount of protein recovered in supernatants (top) and a total amount of protein measured on starches (bottom) are presented. Each value represents a mean of two separate experiments conducted in duplicate. The controls represent baseline values for each starch sample without protein added. There was no difference in control values at different pHs. SDs were less than 10%.

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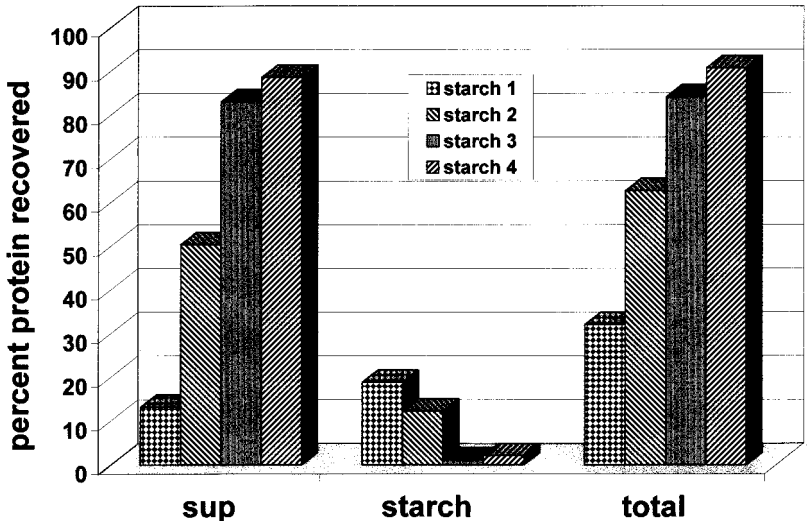

Figure 6. Recovery of protein after starch exposure. Values represent the percentage of total protein (400 μg) recovered in supernatant (sup) and respective starch for each sample exposed to AL and NAL proteins in water.

protein level in a raw material. Variations in glove manufacturing procedures most likely play a role in the amount of protein binding to powder. Lundberg et al.[19] pointed to the importance of the glove manufacturing process as a factor in the final level of protein on powder. It was suggested that starch slurry, if used for a prolonged time, may accumulate a marked amount of "washed off" proteins and consequently, contain a higher level of NRL proteins than for gloves dipped in coating. If that is the case, the evaluation of only a clear extract may not be an accurate reflection of the total protein content, even if taken as a relative indicator.

Evaluation of physical–chemical factors that may contribute to the amount of protein binding to powder binding intensity indicated that binding of NRL proteins to cornstarch powder is dependent of protein concentration, but is not dependent on temperatures in the range of 4–37°C. Higher pH seemed to enhance protein binding in comparison to pH 7.4 or lower. The most striking differences, however, were observed among various types of starch. When four starch samples were compared for their capacity to bind NRL proteins, under the same conditions, the starch powders #3 and #4 showed less affinity for binding NRL protein than the starch powder samples #1 and #2. When water was used instead of the PBS as a binding medium, which is a condition similar to those in manufacturing plants, the differences

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in the binding affinity among starch samples were even more evident. Without the presence of buffer, the pH of powder suspension is higher, which may enhance protein binding, as we observed in our study. In our study, the pH of water was between 6 and 6.4, but with addition of starches #1 and #2, the pH increased up to 9–9.5. In water suspensions of starch #3 and #4, however, pH remained unchanged. This may explain the striking differences among the starch samples exposed to NRL proteins in water, although the corresponding but lesser differences were observed in the pH controlled medium.

Discussing further possible factors affecting protein binding, it appears that the high-binding is not an inherent property of the cornstarch, as one of the low-binding starches was also a cornstarch. On the other hand, both high-binding starch samples #1 and #2 were cross-linked cornstarch dusting powders, while the low binding cooking cornstarch #3 and oat starch dusting powder were not. Based on data presented here, one may associate the high protein binding with a cross-linking process and/or chemicals associated with it. The cross-linking, a procedure needed to prevent hydration of dusting powder in contact with moisture, may create tertiary polysaccharide structures that are more prone to trap proteins. Chemicals used in the process may also change chemical characteristics of the starch, resulting in the increased affinity for protein binding. Common chemicals used for cross-linking include magnesium oxide, phosphorous oxychloride, and epichlorohydrin. It is known that such chemicals are also used for enhancing protein adsorption,[24,25] but the effect seems to be dependent on the presence of those compounds. The cross-linked cornstarch dusting powders should be free of remaining chemicals, but a substantial change of pH when starches were resuspended in water, indicate that it may not be the case. More studies should be performed to determine the specific role of cross-linking in the propensity of starch to bind proteins. Furthermore, starch particle sizes and surface properties may also play a role in binding properties. Oat starch particles are smaller in size ranging from 0.5 to 1.0 μ, while cornstarch particles are larger (1–3 μ).

It is important to note that differences in starch protein levels observed in our study, relate only to the protein remaining on starch after the extraction and washing. Our measurement excluded the extractable protein portion, which is only loosely attached to powder, and is released by water extraction. In the case of starch #1 and #2, we assume that the protein lost by washing the exposed starches may actually represent the extractable protein. On the other hand, earlier studies that evaluated allergen levels on airborne powder,[19–22] measured only the extractable portion. There are no published data that evaluated both, the extractable portions of allergen and the allergen remaining on the powder. The data from different studies cannot be directly correlated because of differences in experimental conditions and allergen quantitation methods. However, based on those reports and the data presented here, it

appears that total allergen on the airborne glove powder includes a water extractable portion and a portion that remains bound to the starch. The reliable estimate of a total amount of allergen on the airborne glove powder should include both.

In summary, our studies show marked differences in the protein binding capacity among various preparations of starches that may be used as glove powders. These laboratory findings may not directly correlate to the large scale production lines. The exposure to proteins in water is similar to the industrial set-up, but other conditions in the manufacturing plants may further affect the binding. We also demonstrated that the proportions of extractable protein from NRL gloves, and the amount of protein remaining on the glove powder vary markedly. Therefore, including the powder fraction into the measurement of protein levels of NRL medical gloves would result in a more accurate measure of their potential allergenicity.

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