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Adsorption of Ovomucoid as Allergenic Protein onto Surfaces

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Abstract. Cleaning of processing equipment in the food manufacturing and of surfaces in catering and food establishments is a key issue in prevention of accidental exposure of individuals with a food allergy to allergens. In this study, ovomucoid was adsorbed onto different surfaces (stainless steel, formica and glass) in various amounts for different periods of time. The results indicated, when ovomucoid was in contact with any of the surfaces, more protein remained on the surface (as determined using the Bradford method) and more immunoreactivity remained (as determined by ELISA) when more protein was put on the surface or when it was left for a longer time. Ovomucoid adsorbed onto stainless steel and formica yielded higher protein remaining and immunoreactivity than was observed for the glass. To our knowledge, this is this the first time that antibody-based methods have been applied for the detection of ovomucoid adsorbed onto different surfaces under various conditions. The results obtained suggest the processors need to be aware of specific problems generated by particular food matrices and the type of surfaces and processes involved.

Keywords: Ovomucoid, Adsorption, Allergenic protein, Surfaces

1. Introduction

Protein adsorption onto solid surfaces has been studied extensively [1]-[4]. However, little systematic work has been carried out on understanding the adsorption of allergenic proteins and the effectiveness or otherwise of cleaning procedures. Adsorption of allergenic proteins on equipment in the food industry and on surfaces used in catering and domestic environments is a key issue in the prevention of accidental exposure of individuals with food allergy to allergenic foods. Adsorption of protein is a very complex phenomenon and, is mediated by various protein-surface forces, such as, hydrophobic, electrostatic, Van der Waals and hydrogen bonding forces. Certain parameters can play an important role in adsorption including pH, temperature, the properties of the surface and of the protein, and also the nature of the solvent. However, the limiting factor in studying allergenic proteins on surfaces is our inability to reliably detect and quantify allergen proteins that may have undergone denaturation, whether through the adsorption process itself or through other relevant factors such as thermal treatment. There are many techniques that can be used for measuring amounts of adsorbed proteins or conformational changes, examples being radiolabeling [5], an enzyme-linked immunosorbent assay [6] and fourier transform infrared spectroscopy [7]. Antibody-based methods are notoriously susceptible to changed responses to different forms of the same protein-despite the commercial availability of diagnostic kits for testing 'swabbed' surfaces for application in the food industry.

In the present work, a specific ELISA-based assay for ovomucoid alongside a non-specific chemical method were used for protein detection to study adsorption of the protein to different surfaces (stainless steel, formica and glass). Protein recovery was affected with the use of cotton swabs. This is the first time that a validated immunochemical method and a chemical assay have been used to investigate the behaviour of allergen proteins on different surfaces.

2. Methods

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2.1. Adsorption of Ovomucoid onto a Surface

Various amounts of ovomucoid (0.2, 0.4 and 0.8 mg/100 µl PBS) were adsorbed onto different surfaces (stainless steel, formica and glass) for different times periods (0hr, 1hr and 15hrs). The samples and surfaces were kept in a desiccator for different time periods.

2.2. Solubilisation Process

Two solubilisations for each amount of ovomucoid were carried out on the same surface for each adsorption time, using two cotton swabs in each of the solubilisation. PBS buffer (pH 7.4) was used as a dilution and extraction buffer to extract ovomucoid particles from the swabs.

2.3. Standards

The Bradford standard curve and non-competitive ELISA standard curve were run for quantifying ovomucoid remaining on the surfaces (direct detection). Both standard curves were run for each assay.

2.4. Detection by Bradford and ELISA Procedures

Direct detection of ovomucoid remaining on surfaces was accomplished *in situ* by using Bradford and ELISA methods.

2.5. Statistical Analysis

The ELISA and Bradford method results were assessed statistically by analysing variance at the significance level (5%) with the LSD test of ANOVA using the SPSS 13.0 version for Windows.

3. Results and Discussion

The first and second solubilisations of swabbed samples were diluted and measured by using Bradford and ELISA methods (the results not shown). A quantification assessment for ovomucoid remaining (after two swabbing steps) on the three surfaces after adsorption of different amounts of ovomucoid for different time periods was carried out by using Bradford procedure and the results are listed in Table 1. The quantitative results confirmed visual assessment by showing that significant amounts of ovomucoid remained adsorbed onto surfaces. Amounts of ovomucoid detected on the three surfaces increased when the adsorption time was longer. These increases in amounts of ovomucoid were significant except between 0hr and 1hr adsorption times. The data in Table 1 demonstrate that there were significant differences in the amounts of ovomucoid detected on the three surfaces after adsorption of three different amounts of protein. The greatest amount of adsorbed ovomucoid (0.8 mg/100µl PBS) gave higher recoveries than the other amounts adsorbed (0.2 or 0.4 mg/100µl PBS).The only significant differences ($p<0.05$) were observed between the recoveries at 0.8 mg/100µl PBS and at both 0.2 and 0.4 mg /100 µl PBS. With regards to the effects of the surfaces on protein adsorption, stainless steel and formica yielded relatively similar amounts of ovomucoid. However, a glass surface yielded much lower amounts than stainless steel and formica surfaces. Statistically, these differences were significant.

A quantification assessment for ovomucoid remaining (after two swabbing steps) on the three surfaces after adsorption of different amounts of ovomucoid for different time periods was carried out by using ELISA procedure and the results are listed in Table 2. The optical density of spot samples was measured and the quantitative results are listed in Table 2. The results show that a higher recovery of ovomucoid was observed after contact with any of the surfaces for a longer adsorption time (15 hrs), and it was a significant quantitative difference ($p<0.05$) in comparison to the shorter adsorption time (0hr) or to an adsorption time of 1hr. Significant differences were also observed between recoveries of ovomucoid for different amounts of ovomucoid adsorbed onto different surfaces, as shown in Table 2. This finding indicates that when a higher quantity of ovomucoid was adsorbed onto the surfaces, there were higher amounts of ovomucoid remaining on the surfaces. From Table 2 it can be seen that ovomucoid remaining on stainless steel was higher than on the other surfaces, though the difference was significant only for stainless steel and glass. Similarly, ovomucoid remaining on formica was greater than that remaining on glass. However, no significant differences were found between ovomucoid remaining on stainless steel and formica. Moreover, the glass surface showed lower resistance of removal ovomucoid from the surface. The major difference between the

antibody-based method (ELISA) and the Coomassie Blue-based method is that the ELISA is based on recognition by an antibody of epitope sites on protein. These epitopes may be affected (increase or decrease of immunoreactivity) by denaturation as result of certain circumstances. Consequently, protein detection would under- or over-estimate amounts of protein, presumably because of steric and configuration changes. In contrast, the Coomassie Blue assay is a colorimetric method for quantifying protein content in different forms and is not affected by conformational changes of the protein.

Table 1: Quantitative results by Coomassie Blue binding for ovomucoid remaining on different surfaces after adsorption of various amounts of ovomucoid for different time periods.

Surface	Time (hr)	Ovomucoid detected directly on surfaces (n=9) (µg/ml)(±STDEV) after two solubilisation steps		
		Adsorption 0.2 mg/0.1ml PBS	Adsorption 0.4 mg/0.1ml PBS	Adsorption 0.8 mg/0.1ml PBS
Stainless Steel	0	2.940 ±0.111	3.129 ±0.122	4.419 ±0.821
	1	3.313 ±0.200	3.667 ±0.380	7.109 ±0.684
	15	6.547 ±0.620	7.800 ±0.341	15.093 ±1.908
Formica	0	2.273 ±0.166	2.552 ±0.211	4.293 ±0.680
	1	2.800 ±0.400	2.948 ±0.186	6.656 ±0.692
	15	5.613 ±0.551	7.413 ±0.310	13.093 ±0.743
Glass	0	BD*	BD	5.181 ±0.569
	1	BD	2.693 ±0.180	5.973 ±0.666
	15	2.573 ±0.271	3.307 ±0.234	8.667 ±0.402

BD = Below the detection limit

Table 2: Quantitative results by ELISA for ovomucoid remaining on different surfaces after adsorption of various amounts of ovomucoid for different time periods.

Surface	Time (hr)	Ovomucoid detected directly on surfaces (n=9) (µg/well)(±STDEV) after two solubilisation steps		
		Adsorption 0.2 mg/0.1ml PBS	Adsorption 0.4 mg/0.1ml PBS	Adsorption 0.8 mg/0.1ml PBS
Stainless Steel	0	0.763 ±0.0179	1.198 ±0.1162	1.765 ±0.2179
	1	0.934 ±0.0483	1.636 ±0.1433	2.174 ±0.1483
	15	1.090 ±0.1024	2.637 ±0.2923	3.654 ±0.2024
Formica	0	0.528 ±0.0155	1.153 ±0.1166	1.349 ±0.1556
	1	0.617 ±0.0172	1.249 ±0.1400	2.038 ±0.1721
	15	0.959 ±0.1111	2.325 ±0.1658	3.373 ±0.1111
Glass	0	0.307 ±0.0152	1.103 ±0.1198	1.157 ±0.1522
	1	0.552 ±0.0880	1.297 ±0.1307	1.439 ±0.1801
	15	0.913 ±0.0431	1.264 ±0.1916	2.296 ±0.2433

In general, the results suggest that significant amounts of ovomucoid remained on the surfaces, even after two successive solubilisation steps. As mentioned earlier, there are many factors that can play a significant role in protein adsorption onto solid surfaces: the concentration and properties of the protein solution, temperature, the pH of the protein solution and the properties of the surface all being examples. The findings show that different concentrations of ovomucoid adsorbed onto different surfaces at room temperature yielded significant quantitative differences in ovomucoid remaining, using both ELISA and chemical methods. Clearly, there was an increase in the amount of ovomucoid that remained adsorbed strongly onto the surfaces when the concentration of ovomucoid solution was increased. Ovomucoid has no free sulfhydryl group (-SH group) to play an important role in adsorption and aggregation of protein by forming intermolecular disulfide linkages. However, the participation of other forces cannot be excluded.

Regarding the temperature of protein adsorption, in the present investigation the temperature was fixed at room temperature. A number of publications [8] [9] indicate that the quantity of proteins adsorbed at elevated temperature (over 65 ℃) is much higher than that adsorbed at room temperature. The denaturation and aggregation of protein often forms multilayers on the surface, in particular in proteins containing cysteine residues. Despite this, significant amounts of protein can adsorb onto surfaces at room temperature. A previous report [9] clarified that the amount of β-lactoglobulin adsorbed onto stainless steel particles increased at first and reached a constant value after 15 minutes at 25℃. In contrast, the present results indicate an increase in the amount of adsorbed ovomucoid after leaving onto surfaces for a prolonged period of time (15hrs) at 25 ℃. The possible reason for this may be related to the differences in the properties of the protein and/or experimental conditions.

Further research may be required into the physico-chemical properties of stainless steel, formica and glass surfaces, and their hydrophilicity and hydrophobicity, for example, to which could be ascribed the participation of the electrostatic and hydrophobic interactions in the current work.

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