

Interaction between protein allergens and model gastric emulsions

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Abstract

The observed resistance to pepsinolysis of known food allergens has been suggested as a predictor of their allergenic risk. Consequently, resistance to pepsinolysis has become incorporated into decision tree assessment for potential allergenic risk posed by novel foods. However, existing methods take little account of the interaction between food structure and physiological conditions existing during digestion *in vivo*. Here we show that a range of protein allergens can adsorb to model stomach emulsions, providing a further means of resisting digestion. We also show that raising the pH and the addition of bile salts to a model stomach emulsion, thereby mimicking the duodenal environment, has the effect of desorbing the adsorbed protein.

Introduction

The mechanisms by which food allergens sensitize an individual via the gastrointestinal tract are currently unclear. The harsh conditions of the gastrointestinal tract (low pH, proteolytic enzymes and surfactants such as phospholipids and bile salts) favour rapid and extensive hydrolysis of proteins, yet food protein allergens retain sufficient structural integrity to be taken up by the gut and sensitize the mucosal immune system. While it has been demonstrated that allergens generally have a remarkable resistance to pepsinolysis [1], the current models incorporated in the proposed decision tree assessment of allergenic risk [2] do not consider the multi-phase nature of the digestive processes in the stomach. This environment could be highly relevant, since many plant food allergens have an ability to bind lipids and associate with membranes. For example β -lactoglobulin (β -lg), a potent allergen from

cow's milk, binds palmitate strongly [3]. Furthermore, the 2 S albumin family of storage proteins, which includes major allergens from yellow mustard seed (Sin a 1 [4]), peanut (Ara h 2 [5]) and Brazil nut (Ber e 1 [6]), and SFA-8 (methionine-rich 2 S albumin 8 from sunflower) [7], are able to bind or associate with lipids. However, this is not through the action of a binding pocket, as is the case for members of the non-specific lipid transfer protein family, but through their activity as efficient emulsifiers, as shown for SFA-8 from sunflower [8]. Sin a 1 has also been shown to associate with phospholipid lipid vesicles [9], perturbing the bilayer and causing the formation of leaky structures. Other food protein allergens, such as those belonging to the cereal-inhibitor family, may also associate with lipids. While normally associated with respiratory allergies such as Baker's asthma, members of this protein family have also been found to cause orally sensitized allergies, including an $M_r \sim 15000$ inhibitor subunit involved in wheat allergy [10]. Recently we have found that a member of this family, CM3, also has the ability to interact with lipids (G. R. Burnett, unpublished work). Such lipid-associated proteins may be protected from the proteolytic environment in the gut, and there is evidence that proteins associated with lipid bilayers have enhanced thermal stability [11]. Lipid binding may also assist allergen absorption by the gut mucosa, either through association with cell membranes, as has been suggested for 2 S albumins [9], or by co-adsorption on the surface of lipid droplets. Furthermore, β -lg adsorbed to emulsion interfaces appears to show resistance to tryptic digestion [12], and the pattern of peptides obtained during digestion differs from that for β -lg in solution.

In this paper we demonstrate how proteins may avoid enzymic digestion in the gastric environment by adsorbing to emulsion droplets.

Methods

Model stomach emulsions were prepared by sonicating 0.74 mM lecithin (Lipid Products;

Key words: pepsinolysis, protein-lipid interaction, protein adsorption.

Abbreviations used: β -lg, β -lactoglobulin; SFA-8, methionine-rich 2 S albumin 8 from sunflower.

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1 mg/ml protein) with *n*-hexadecane (Sigma; > 99%) and buffer (50 mM sodium citrate/citric acid, pH 2.5). The proteins used were horse myoglobin, bovine β -lg, bovine β -casein and BSA (all used as supplied by Sigma), CM3 (purified as in [10]), Ber e 1 (prepared as in [13]) and sunflower seed proteins (SFA-8 and SF-lipid transfer protein; prepared as in [8]).

Model duodenal emulsions were prepared from a model stomach emulsion containing 10% (v/v) *n*-hexadecane and 1 mg/ml β -lg by adjusting the pH to 6.5 with NaOH and adding a bile salt mixture containing equimolar quantities of taurocholic acid sodium salt (Fluka) and glycodeoxycholic acid (Calbiochem).

The amount of non-adsorbed protein in the model gastric emulsion was subsequently determined 2 h after emulsion preparation. Each emulsion was centrifuged at 5000 *g* for 5 min so that the emulsion phase separated into a cream and a sub-phase. This sub-phase, containing the non-adsorbed protein, was analysed by the bicinchoninic acid method [14]. Each measurement is the average of three separate analyses on at least two different emulsions.

Results and discussion

The amount of non-adsorbed protein present in the sub-phase of model stomach emulsions as a function of the oil volume fraction is shown in Figure 1. All of the proteins used in the present study (including the non-allergenic myoglobin) adsorbed to the emulsion interface, showing depletion in the sub-phase. It is also apparent that, as the oil volume increased, the amount of protein that was adsorbed to the emulsion interface increased. This would suggest a deficiency in the amount of surfactant material present in the model emulsion, since surfactants generally displace proteins from interfaces. While the amount of phospholipid used in this study was small (0.74 mM), this is typical of the concentration in the gastric secretions, so it would appear that the stomach is relatively deficient in surfactants. This conclusion is supported by the fact that phase separation in the stomach has been observed in human subjects after eating lipid-rich meals (M. Wickham, unpublished work). However, even after increasing the phospholipid concentration to 5 mM in these model emulsions, significant protein adsorption is still observed (G. R. Burnett, unpublished work). Furthermore, preliminary results suggest that pepsin digestion of proteins

is retarded when they are incorporated into these model stomach emulsions, and that these proteins show a degree of secondary structural change

Figure 1

Adsorption of protein to model stomach emulsions as a function of the volume of oil

Proteins used were β -lg ($\square$), BSA ($\circ$), SFA-8 ($\triangle$), sunflower lipid transfer protein (∇), Ber e 1 ($\blacksquare$), CM3 ($\bullet$), myoglobin ($\blacktriangledown$) and β -casein ($\blacktriangle$). Lines are a guide to the eye only.

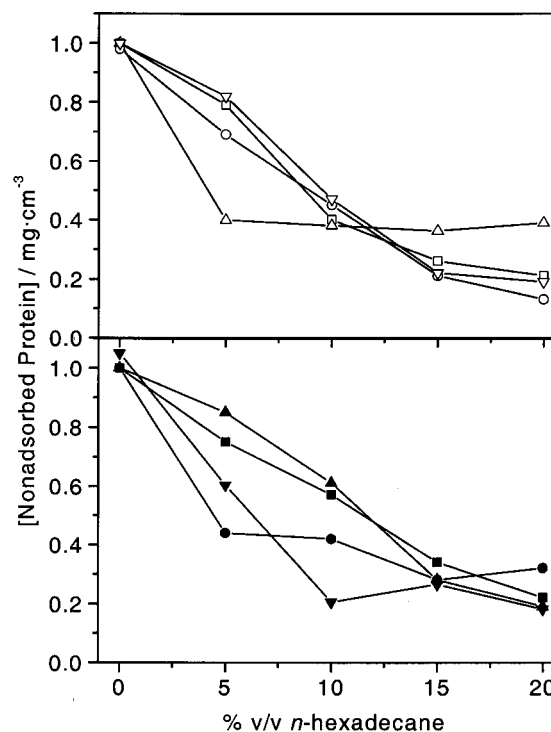
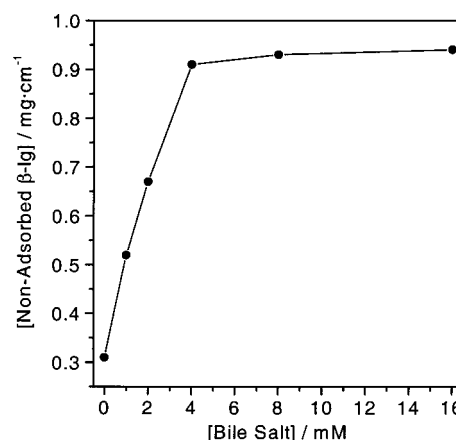


Figure 2

Desorption of β -lg from a model stomach emulsion by the addition of bile salt

The model emulsion contained 1 mg/ml β -lg and 10% (v/v) *n*-hexadecane. Lines are a guide to the eye only.



when incorporated at an emulsion interface (G. R. Burnett, unpublished work).

The effect of changing the properties of the model emulsion to reflect those found in the duodenum (raising the pH to 6.5 and the addition of bile salts) can be seen in Figure 2. Essentially, bile salt addition removed the adsorbed protein from the emulsion, resolubilizing it in the continuous aqueous phase. This effect was particularly marked in the case where large concentrations of bile salts were used. However, even at physiological concentrations of bile salts ($\sim 4\text{--}5\text{ mM}$), 90% of the protein that was adsorbed in the stomach emulsion was desorbed in the presence of bile salts. The overall effect of adsorption in the stomach followed by desorption in the duodenum would be to protect the protein from pepsinolysis and subsequently expose it to pancreatic proteases. This would suggest a potential flaw in the current models for assessing potential allergenicity, which rely extensively on resistance to pepsinolysis. Moreover, it is probable that the desorbed protein is denatured and bound to surfactants (possibly even associated with the mixed micelles that are present in the duodenum), further complicating enzymic digestion.

While we acknowledge that the systems utilized in the present study are model systems, lacking the complexity of *in vivo* digestive processes, it is clear that the multi-phase nature of the gastric environment could play an important role in presenting allergenic proteins to the immune system, and that current risk assessments for allergenicity could be oversimplified.

IACR and the Institute of Food Research receive grant-aided support from the Biotechnology and Biological Sciences Research

Council (BBSRC), U.K. The present work was also supported by BBSRC grant 233/D10117.

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Received 18 June 2002