

Xaver Baur

Enzymes as occupational and environmental respiratory sensitisers

Received: 29 July 2004 / Accepted: 12 November 2004 / Published online: 8 April 2005
© Springer-Verlag 2005

Abstract A literature review shows that airborne enzymes occurring in the general environment and in purified form in industrial production have a high allergenic potential to the airways, causing rhinitis, conjunctivitis and asthma. It can be assumed that this also applies to the increasing number of enzymes manufactured by the cloning of fast-growing genetically engineered microorganisms. Cross-sectional studies demonstrate exposure–response relations for IgE-mediated sensitisation and airway disorders. Atopic individuals are more susceptible to enzyme allergy than non-atopic individuals. Skin prick testing and measurement of specific IgE antibodies have been shown to be useful diagnostic tools. Very high concentrations of proteases may lead to emphysema. There is also evidence for non-allergic airway inflammation by proteases, probably via protease-activated receptor-2 and intracellular Ca^{2+} release. It is recommended that all enzymes be classified with the risk phrase R42 (may cause sensitisation by inhalation) and that their inhalative uptake be totally avoided.

Keywords Enzymes · Occupational asthma · Rhinitis · Allergy · Prevention

Introduction

The allergenic potency of airborne enzyme dust aroused major medical and media interest in the late 1960s and

the 1970s when alkaline heat-stable enzymes such as the protease subtilisin, amylases and cellulases were introduced into the detergent industry (Flindt 1969; Wüthrich and Ott 1969; Newhouse et al. 1970; Weill et al. 1971; Musk and Gandevia 1976; Juniper et al. 1977). As mentioned in more detail in the section “Exposure–response relationship”, at that time up to 50% of the workers developed hypersensitivity reactions. Nowadays, a large number of enzymes are produced and used by the industry. An increasing number of them is genetically engineered and modified with the help of recombinant DNA technology. Their allergenic potential is mostly unknown. Besides their existence in certain workplaces, enzymes are also present in the general environment as natural components of many plant and animal sources and are responsible for a significant number of allergies such as rhinitis and bronchial asthma in the population. In this paper, present knowledge of health risks and disorders caused by airborne enzymes are summarised and appropriate preventive measures are discussed.

Nomenclature and classification of enzymes on the basis of their catalysed reactions

The Enzyme Commission of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB 2004) has enlisted and updated enzymes according to their type of catalysed reactions or reacting groups, e.g. hydrolases, which cleave polymeric substrates by the addition of water. Among others, hydrolases comprise proteases, carbohydrases and ribonucleases (Table 1).

Enzymes as natural allergenic components in the environment

As summarised in Table 2, being natural components of plants and animals, enzymes, represent major allergens

X. Baur
Institute of Occupational Medicine,
University of Hamburg,
Hamburg, Germany

X. Baur
Ordinariat und Zentralinstitut für Arbeitsmedizin Hamburg,
Seewartenstrasse 10, 20459 Hamburg, Germany
E-mail: baur@uke.uni-hamburg.de
Tel.: +49-40-428894501
Fax: +49-40-428894514

Table 1 Classes and some subclasses of enzymes according to the Enzyme Commission (EC) nomenclature (NC-IUBMB 2004)

Enzyme class
Oxidoreductases (EC 1.-.-.-)
Transferases (EC 2.-.-.-)
Hydrolases (EC 3.-.-.-)
Esterhydrolases (EC 3.1.-.-)
Glycosidases (EC 3.2.-.-)
Ribonucleases (EC 3.3.-.-)
Proteases (peptidohydrolases (EC 3.4.-.-)
Serine proteases (serine endopeptidases, EC 3.4.21.-)
Cysteine proteases (cysteine endopeptidases, EC 3.4.22.-)
Aspartate proteases (aspartate endopeptidases, EC 3.4.23.-)
Metalloproteases (metalloendopeptidases, EC 3.4.24.-)
Lyases (EC 4.-.-.-)
Isomerases (EC 5.-.-.-)
Ligases (EC 6.-.-.-)

in a variety of outdoor or indoor dust sources including occupational ones.

Purified enzymes causing allergy in industrial workers

Table 3 gives an overview of the major enzymes or enzyme groups produced, isolated and used in modern industry. Nearly all of them have been reported to cause respiratory and other allergies in occupationally exposed subjects (Table 4).

Probably the largest occupational group endangered by airborne enzymes is bakers. A recent prospective study by Brisman et al. (2004) showed that, within a period of 3 years, 8% developed IgE antibodies to α -amylase; many of them suffered from eye/nose and/or chest symptoms.

A prospective follow-up investigation of 1,064 workers in a large enzyme-producing company revealed

Table 2 Examples of natural allergenic enzyme components in indoor or outdoor dust sources

Indoor/outdoor source	Allergen ^a
House dust mites, <i>Dermatophagoides pteronyssinus</i> , <i>Dermatophagoides farinae</i> , <i>Dermatophagoides microceras</i>	Der f 1, Der p 1, Der m 1 (cysteine protease), Der p 3, Der f 3 (trypsin) Der p 4 (amylase) Der p 6 (chymotrypsin) Der f 15 (98 k chitinase) Der f 18 w (60 k chitinase) Eur m 1 (cysteine protease) Blo t 1 (cysteine protease) Blo t 3 (trypsin) Blot 4 (α -amylase) Blot 6 (chymotrypsin) Blot 6 (chymotrypsin) Bla g 2 (inactive aspartic protease) Asp f 5 (metalloprotease) Asp f 6 (manganese superoxide dismutase) Asp f 10 (aspartic protease) Asp f 13 (alkaline serine protease) Asp f 18 (vacuolar serine protease) Asp o 13 (alkaline serine protease) Asp o 21 (TAKA-amylase A) Asp f 22 w (enolase) Cyn d 22 w (enolase) Cas s 5 (chitinase)
Mite, <i>Euroglyphus mayne</i> Mite, <i>Blomia tropicalis</i>	
German cockroach <i>Aspergillus fumigatus</i>	
<i>Aspergillus oryzae</i>	
Bermuda grass Chestnut	
Occupational sources Honey bee	Allergen Api m 1 (phospholipase A2) Api m 2 (hyaluronidase) Api m 7 (CUB serine protease) Trypsin, α -amylase, lipase Chit 1–9 (haemoglobin) Peroxidase Hev b 2 (1,3-glyconase) Hev b 9 (enulase) Hev b 10 (manganese superoxide dismutase) Hev b 11 (class 1 chitinase) Hev b13 (esterase) Bromelaine Papain Trip s 1 (class 1 chitinase) Hor v 16 (α -amylase) Hor v 17 (β -amylase)
Pancreatin drugs Midge, <i>Chironomus thummi thummi</i> Wheat flour Natural rubber latex, <i>Hevea brasiliensis</i>	
Pineapple Papaya fruit Obeche wood Barley	

^aInternational Union of Immunological Societies (2004)

Table 3 Examples of enzymes or enzyme groups used in industry. According to AMFEP (2001), 186 commercial enzymes were manufactured in the EU, 158 for the food industry, 64 for technical application and 57 for feedstuffs

Enzyme/ enzyme group	Industrial branch							
	Pharmacy	Dairy	Bakery	Catering/ food industries	Feed industry	Paper industry	Detergent industry	Textile industry
Proteases (EC 3.4) ^a	X	X		X	X		X	X
Amylases (EC 3.2.1)			X	X		X	X	X
Lipases (EC 3.1.1)	X	X		X	X		X	X
Lysozymes (EC 3.2.1.17)	X		X					
Cellulases (EC 3.2.1.4)			X	X	X	X	X	X
Hemicellulases (EC 3.2.1.8)			X			X		
Xylanases (EC 3.2.1.8)			X	X	X	X		X
Glucoamylases (EC 3.2.1.3)			X					
Pectinases (EC 3.2.1.15)				X				
Lactases (EC 3.2.1.23)	X							
Catalases (EC 1.11.1.6)	X							
Phytase (EC 3.1.3.8)					X			
Hyaluronidases (EC 3.2.1.35; 4.2.2.1)	X							X

^aMore than 1,000 tonnes/year

that 5% had work-related asthma and 3% rhino-conjunctivitis; IgE-mediated sensitisation mainly occurred to subtilisin, glycoamylase and/or trypsins (Johnsen et al. 1997).

Among 29 cooks in an industrial kitchen where a papain-containing meat-tenderiser was used, ten (34.5%) were sensitised and 50% reported respiratory and/or conjunctival hypersensitivity symptoms (Baur et al. 1982).

Example of a cross-sectional study in an enzyme (phytase)-confectioning plant

We recently studied 53 subjects occupationally exposed to powdered phytase from *Aspergillus niger*, which has become a widely used animal feed additive (Baur et al.

2002). This enzyme, a phosphatase, increases the bio-availability of phosphorus of plant origin in animal feeds. It catalyses the hydrolysis of phytase to lower-order phosphate esters and to inorganic phosphate and is used mainly for pigs and poultry, i.e., monogastric animals. A total of 43% of the workers were heavily exposed to phytase dust in large-scale confection, whereas 57% worked in low-exposure areas such as laboratories and experimental animal husbandry. All subjects were interviewed by the company physician and had their phytase-specific IgE antibody levels measured.

A total of 87% of the heavily exposed and 60% of the low-exposure subjects complained of work-related symptoms ($P=0.031$), mainly dyspnoea ($n=12$), rhinitis ($n=35$), conjunctivitis ($n=16$) and/or cutaneous hypersensitivity ($n=3$).

Fig. 1 Cross-sectional study of 53 workers occupationally exposed to phytase from *Aspergillus niger*: study group, symptoms and results of phytase-specific IgE measurements (Baur et al. 2002)

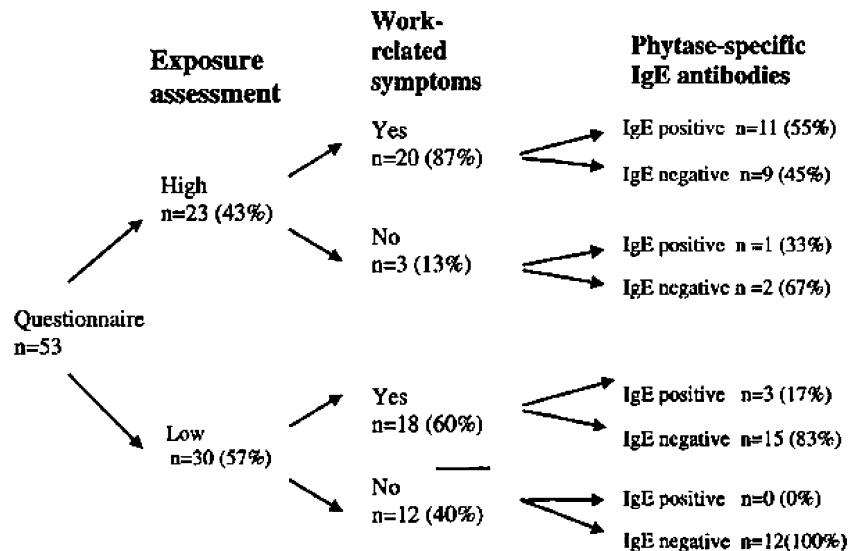


Table 4 Examples of occupational allergies caused by airborne enzymes (*A* asthma, *R* rhinitis, *C* conjunctivitis, *Cu* cutaneous symptoms)

Enzymes (source) EC-number	No. of subjects (study design)	Symptoms (%) of exposed workers	Specific IgE antibodies	References
Phytase (<i>Aspergillus niger</i>) EC 3.1.3.8	53 (cross-sectional)	A (23%), R (68%), C (31%), Cu (6%)	32%	Baur et al. (2002)
α -Amylase (<i>Aspergillus oryzae</i>) EC 3.2.1.1	171 (symptomatic workers) 261 (symptomatic workers) 300 (prospective)	A, R, C A, R, C A (11%), R/C (24%)	23% 23% 8% ^b	Sander et al. (1998) Baur et al. (1989) Brisman et al. (2004)
Glucoamylase (<i>Aspergillus niger</i>) EC 3.2.1.3	171 (symptomatic workers) 261 (symptomatic workers) 1,064 (retrospective)	A, R, C A, R, C A (5%), R/C (3%) ^b	8% 5% 11%	Sander et al. (1998) Baur et al. (1989) Johnsen et al. (1997)
Cellulase (<i>Aspergillus niger</i>) (<i>Trichoderma reesei</i>) EC 3.2.1.4	171 (symptomatic workers) 365 (cross-sectional)	A, R, C A (16%), R, Cu	13% 2% ^b	Sander et al. (1998) Strassburger et al. (1999)
Xylanase (<i>Aspergillus niger</i>) EC 3.2.1.8	171 (symptomatic workers)	A, R, C	11%	Sander et al. (1998)
Lactase (<i>Aspergillus</i> spp.) EC 3.2.1.23	207 (cross-sectional)	A (10%), R (20%), C (9%)	31%	Muir et al. (1997)
Hemicellulase (<i>Aspergillus niger</i>) EC 3.2.1.78	261 (symptomatic workers)	A, R, C	10%	Baur et al. (1989)
Trypsin (bovine pancreas) EC 3.4.21.4	1,064 (retrospective)	A (5%), R/C (3%) ^b	11%	Johnsen et al. (1997)
Subtilisin	1,064 (retrospective)	A (5%), R/C (3%) ^b	16%	Johnsen et al. (1997)
Subtilisin A Esperase EC 3.4.21.62	1,064 (retrospective)	A (5%), R/C (3%) ^b	22%	Johnsen et al. (1997)
Savinase (<i>Bacillus licheniformis</i> ; <i>Bacillus subtilis</i>)	1,064 (retrospective)	A (5%), R/C (3%) ^b	5%	Baur et al. (2000)
Papain (<i>Carica papaya</i>) EC 3.4.22.2	29 (cross-sectional)	A (45%), R (42%), C (15%), Cu (10%)	40%	Wüthrich and Ott (1969)
Bromelain (<i>Ananas comosus</i>) EC 3.4.22.23	10 (cross-sectional)	A (40%), R (30%), Cu (30%)	50%	Kämpe et al. (1989)
Collagenase (<i>Clostridium histolyticum</i>) EC 3.4.24.3	39 (cross-sectional)	A (15%), R, C, Cu	n.d.	

^aCo-exposure with other enzymes^bSkin prick test

Fourteen of the 38 symptomatic subjects and one asymptomatic worker were sensitised. All but one of the sensitised employees were symptomatic. The frequency of IgE-positive subjects was 52% in the high-exposure group and 10% in the group with low exposure (12/23 vs 3/30; $P=0.001$) (Fig. 1).

However, there was no significant relationship between the levels of IgE antibodies and the degree of exposure.

In immunoblots, IgE-positive patients showed mainly IgE binding to a 52 kDa protein of deglycosylated phytase, which represented *A. niger* phytase. Minor IgE binding to proteins of ca. 40 kDa and 30 kDa was also seen.

In summary, those results demonstrated that powdered fungal phytase is—as are many airborne enzymes—a highly sensitising substance whose inhalative uptake should be totally avoided. Our follow-up investigation, after installation of a local exhaust system and the wearing of all-protective clothing and masks with FFP 2 filters by all employees, resulted in an immediate cessation of hypersensitivity symptoms.

New genetically engineered enzymes as potential allergens

It is estimated that approximately 100 genetically engineered enzymes are already on the market. Table 5 summarises 30 types of enzymes produced from genetically modified fast-growing microorganisms (AMFEP 2001). It can be assumed that many of them have encoded modified enzyme genes expressed for commercial use. The methods applied for the production of genetically engineered enzymes include:

- oligonucleotide-directed mutagenesis (modification of one or a few amino acids),
- DNA (gene) shuffling, i.e., combinations of two or more fragments of genes from various sources possessing different useful functions; examples are α -amylases, lipases, subtilisins, esterases, dehydrogenases,
- introduction of active enzyme sites into a protein fragment scaffold.

It should be mentioned that the allergenicity of genetically engineered enzymes in humans is mainly unknown. Nevertheless, due to their structural relationship with allergenic native enzymes there is evidence of allergenic potency. It may be that the genetic homogeneity of this material lowers the numbers of allergenic sites but potentially increases their concentration.

Enzymes used in food processing do not have to be listed. Also, when enzymes are genetically engineered, i.e. structured and produced through recombinant DNA technology, the manufacturer is not required to notify the FDA or EU bodies that the enzyme has been modified.

Table 5 Types of enzymes from genetically modified microorganisms (AMFEP 2001)

Enzyme	
Acetolactate decarboxylase (alpha)	Lipase
Aminopeptidase	Maltogenetic
Amylase (alpha)	Mannanase amylase
Arabinofuranosidase	Pectate lyase
Catalase	Pectin lyase
Cellulase	Pectin methylesterase or pectinesterase
Chymosin	Penicillin amidase
Cyclodextrin glucotransferase	Phospholipase A
Galactosidase (alpha)	Phospholipase B
Gluconase (beta)	Phytase
Glucoamylase or amyloglucosidase	Polygalacturonase or pectinase
Glucose isomerase	Protease (including milk-clotting enzymes)
Glucose oxidase	Pullanase
Hemicellulase	Xylanase
Laccase	
Lactase or galactosidase (beta)	

Exposure–response relationships

A few studies have investigated exposure–response relationships (Baur et al. 2002; Brisman et al. 2004; Sander et al. 1998; Vanhanen et al. 1996, 1997; Houba et al. 1996). In the latter investigation, of 178 Dutch bakers, a positive association between the air concentration of α -amylase in bakeries and the frequency of sensitisation (starting at concentrations as low as 0.25 ng/m³) was found. This effect was much more pronounced in atopic patients than in non-atopic patients (Fig. 2).

Corresponding findings were recently reported in 300 British bakers and millers, with a significant relationship between sensitisation and symptoms (Brisman et al. 2004).

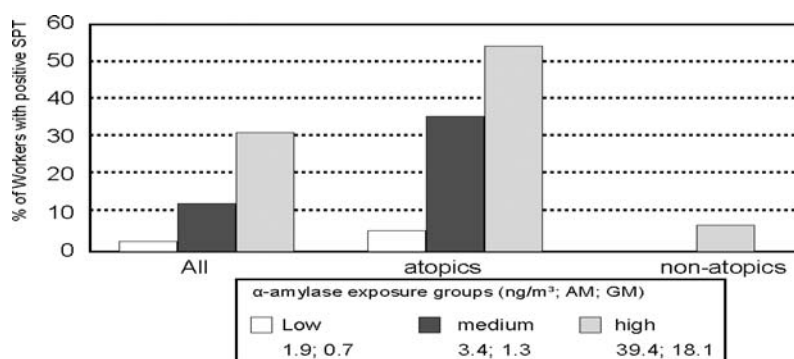
In the detergent industry, enzyme workplace concentrations in the late 1960s were approximately 300 ng/m³; at that time, approximately 40%–50% of the workers were sensitised and developed asthma and/or rhinitis. At present, owing to enzyme encapsulation and other primary and secondary preventive measures, exposures have been reduced mainly to <15 ng/m³. This decrease is reported to be associated with less than 20% sensitisation during a period of 10 years and an annual incidence of less than 3% (Schweigert et al. 2000). Cullinan et al. (2000) found,

in a nested case–control analysis, that 19% of detergent workers (geometric mean concentration 4.25 ng/m³) were sensitised and that 16% complained of work-related respiratory symptoms. In this study, seven of a subgroup of 74 subjects who had started work 3 years ago were sensitised (five to protease and/or amylase and four to cellulase) and five had noticed work-related symptoms of the upper respiratory tract and/or of the chest.

Amplification of allergies and direct induction of inflammatory reactions by exogenous proteases of natural sources

There is evidence that at least proteases have, in addition to their allergenic potency, direct effects on epithelial integrity and permeability, mast cell degranulation, cytokine release from the respiratory epithelium and, further, exhibit interactions which may influence the pathogenesis of allergic and non-allergic airway disorders (Stewart et al. 1993). Recent studies show that proteases can react with cell surface receptors (protease-activated receptors) in the airways. This leads to leukocyte infiltration and gene transcription, which produces integrins, chemokines, cytokines and cyclooxygenase 2.

Fig. 2 Exposure–response-relationship in 49 atopic and 84 non-atopic bakers with various exposure levels of α -amylase of *Aspergillus oryzae* (Houba et al. 1996). SPT immediate-type skin prick test response, AM arithmetic mean, GM geometric mean. Left bar low (1.9; 0.7), centre bar medium (3.4; 1.3), right bar high (39.4; 18.1)



Enzymes from tree and grass pollen were found to cause lesions of airway epithelial cells of mice (Hassim et al. 1998).

Der p 1, a cysteine protease from the house-dust mite *Dermatophagoides pteronyssinus*, which accounts for approximately 20% of the total protein content of mite faecal pellets (ca. 0.1 ng/pellet), facilitates its own passage (along with other allergens) across the epithelium and gains access to cells of the immune system. Der p 1 can cleave, proteolytically, cell surface molecules involved in the homeostatic control of IgE synthesis, namely CD40 on dendritic cells, CD25 on T cells and CD23 on B cells. It also enables the differentiation of naive T cells towards the Th2 phenotype and IgE synthesis. Furthermore, Gough et al. (2003) demonstrated that the administration of proteolytically active Der p 1 to mice through the intranasal route leads to significant inflammatory cellular infiltration of the lungs and to the systemic production of IgE. The animals had been initially sensitised with proteolytically active Der p 1 through intraperitoneal and subcutaneous routes and subsequently boosted intranasally. Finally, they were intranasally challenged by proteolytically active Der p 1, inactive Der p 1 or phosphate buffer solution.

Proteases, among others, also enhance the availability of the antigen by antigen-presenting cells (Gough et al. 2003).

Asp f 13, an alkaline serine proteinase from *Aspergillus fumigatus*, was shown to be involved in airway inflammation and remodelling (Kurup et al. 2002).

On the molecular basis, Der p 1, 3 and 9 induce cytokine production by airway epithelial cells, which is partially mediated by the protease-activated receptor-2 (Asokanathan et al. 2002), resulting in non-allergic inflammation.

Hong et al. (2004) found that German cockroach extract with known protease activity also stimulated protease-activated receptors in human epithelial cells and resulted, intracellularly, in the generation of $[Ca^{2+}]$ oscillation through Ca^{2+} release from Ca^{2+} stores.

Emphysema caused by high air concentrations of proteolytic enzymes

It should be mentioned that—as shown in animal experiments—inhalative uptake of high concentrations of airborne proteases is associated with emphysematous lesions of the lungs (Gross et al. 1964, 1965; Palecek et al. 1967; Goldring et al. 1968; Ackerman et al. 1977).

Workers highly exposed to papain-containing dust also provided evidence of emphysema (Gandevia and Mitchell 1971). Musk and Gandevia (1976) reported loss of pulmonary elastic recoil in detergent workers. Kizkin et al. (2002) found reduced values of forced vital capacity (FVC) and forced expiratory volume in one second (FEV)₁ and respective annual declines in protease-sensitised detergent workers.

Regulations

According to EEC directive 62/548, only 16 delta-glucosidase, cellulase, exo-cellulohydrolase, alpha-amylase, other amylases, ficin, papain, pepsin A, rennin, trypsin, chymotrypsin, microbial neutral proteinase, other proteinases, bromelain juice and subtilisin, of the approximately 200 commercially available enzymes, are classified with the risk phrase R42 (may cause sensitisation by inhalation); nine of them are additionally classified R 36/37/38 (irritating to eyes, respiratory system and skin); one (subtilisin) is also classified R41 (risk of serious damage to eyes). In Europe, no threshold limit value/occupational exposure limit (TLV/OEL) exists; in the USA only one TLV is provided by the American Conference of Governmental Industrial Hygienists (ACGIH) (2004), namely for subtilisin (60 ng/m³), which seems to be much too high under a health-based consideration.

In Germany, the above mentioned EEC directive 67/548 is in force. The latest maximale Arbeitsplatzkonzentration (MAK; maximal workplace concentration) and biological tolerance (BAT biological workplace concentration) values, for 2004, (Deutsche Forschungsgemeinschaft 2004) designate the following enzymes as Sa (danger of sensitisation to airways):

- α -amylase
- cellulases
- subtilisin
- xylanase

Several additional enzymes are currently being examined for inclusion in this list of MAK and BAT values as airway sensitisers.

Discussion

There is evidence that purified hydrolytic (EC group 3) enzymes in particular, but also other enzymes, behave as allergens when inhaled. The prevalence of sensitisation and symptoms among occupationally exposed subjects is mostly in the range between 5% and 20% and may reach 50% under bad hygiene conditions. However, enzymatic activity is not a prerequisite for allergenicity; several allergen groups such as enzyme-inhibitors, transport proteins and regulatory protein lectins do not show enzymatic activity (Stewart and McWilliam 2001).

Recent data confirm on a molecular level that, in addition to IgE-mediated sensitisation, proteases cause non-allergic inflammation in the airways via protease-activated receptor-2 in respiratory epithelial cells. In this connection, the detected increased chitinase expression in airways of asthmatics is remarkable (Zhu et al. 2004). These results indicate a pathophysiological role of proteases in driving the allergenic response and non-allergic inflammation of airways.

At very high concentrations of proteases, epithelial and emphysematous lesions may occur. With regard to this finding, it is worth mentioning that even endogenous proteases can cause emphysema in the case of hereditary $\alpha 1$ -protease inhibitor deficiency (ZZ or SZ phenotypes).

The presented data demonstrate that, in spite of exposure reduction in several industries, i.e. in detergent production, a rather high number of sensitisations and respiratory allergies exists. This indicates that stricter regulations for powdered enzymes are necessary. All enzymes should be classified as R42, and inhalative exposure must be totally avoided. Furthermore, more detailed studies of exposed workers are necessary to define exposure–response relationships more precisely. All existing data on exposure assessment and medical surveillance of exposed workers should be disclosed, and independent risk and TLV/OEL assessment is required. This is now possible, since sensitive and specific immunoassays for quantification of airborne enzymes have recently been developed (Heederik 2003).

In spite of a lack of information on some enzymes, it can be assumed that all airborne enzymes occurring in certain workplaces and at much lower concentrations in the general environment behave as potent respiratory allergens in a dose-dependent manner. This means that reducing the respiratory air load reduces the health risk, e.g. by the enclosure of processes, utilisation of hoods and local exhaust systems, adequate ventilation, and use of less-dusty preparations and respiratory protection equipment. Dermal contact and inoculation may also be a significant risk factor for induction of IgE-mediated cutaneous and respiratory allergy and should be taken into consideration for preventive measures. Therefore, limitation of exposure remains a continuous task. Further important preventive measures in the industry include detailed information of employees, employers and occupational health professionals, availability of material safety data sheets indicating the enzyme content of products and their health risk, industrial health programmes for systematic minimisation of exposure and regular medical surveillance of endangered workers.

References

- Ackerman NR, Corkey R, Perkins D (1977) Pathogenesis of papain emphysema in the hamster. *Am Rev Respir Dis* 4:199
- Asokanathan N, Graham PT, Stewart DJ, Bakker AJ, Eidne KA, Thompson PJ, Stewart GA (2002) House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J Immunol* 169:4572–4578
- Association of Manufacturers and Formulators of Enzyme Products (AMFEP) (2001) List of commercial enzymes. Updated 1 October 2001. Brussels. <http://www.amfep.org>
- Baur X, König G, Bencze K, Fruhmans G (1982) Clinical symptoms and results of skin test, RAST and bronchial provocation test in thirty three papain workers: evidence for strong immunogenic potency and clinically relevant “proteolytic effects of airborne papain”. *Clin Allergy* 12:9–17
- Baur X, Sauer W, Weiss W, Fruhmans G (1989) Inhalant allergens in the modern baking industry. *Immunol Allergy Pract* 11:13–15
- Baur X, Sander I, van Kampen V (2000) Aerogene Enzyme sind aggressive berufliche Inhalationsallergene. *Dtsch Med Wochenschr* 125.30:912–917
- Baur X, Melching-Kollmuss S, Koops F, Strassburger K, Zober A (2002) IgE-mediated allergy to phytase—a new animal feed additive. *Allergy* 57:943–945
- Brisman J, Nieuwenhuijsen MJ, Venables KM, Putcha V, Gordon S, Taylor AJ (2004) Exposure–response relations for work related respiratory symptoms and sensitisation in a cohort exposed to alpha-amylase. *Occup Environ Med* 61:551–553
- Cullinan P, Harris JM, Newman Taylor AJ, Hole AM, Jones M, Barnes F, Jolliffe G (2000) An outbreak of asthma in a modern detergent factory. *Lancet* 356:1899–1900
- Deutsche Forschungsgemeinschaft (2004) List of MAK and BAT values 2004. Wiley-VCH, Weinheim
- Flindt MLH (1969) Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet* 1:1177–1181
- Gandevia B, Mitchel C (1971) The dangers of proteolytic enzymes to workers. *Med J Aust* 1:1032
- Goldring I, Greenburg L, Ratner I (1968) On the production of emphysema in Syrian hamsters by aerosol inhalation of papain. *Arch Environ Health* 16:59–60
- Gough L, Campbell E, Bayley D, Van Heeke G, Shakib F (2003) Proteolytic activity of the house dust mite allergen Der p 1 enhances allergenicity in a mouse inhalation model. *Clin Exp Allergy* 33:1159–1163
- Gross P, Babyak A, Tolker E, Kaschak M (1964) Enzymatically produced pulmonary emphysema. *J Occup Med* 6:481
- Gross P, Pfitzer EA, Tolker E, Babyak MA, Kaschak M (1965) Experimental emphysema. *Arch Environ Health* 11:50–58
- Hassim Z, Maronese SE, Kumar RK (1998) Injury to murine airway epithelial cells by pollen enzymes. *Thorax* 53:368–371
- Heederik D (2003) Allergen exposure and occupational respiratory allergy and asthma. In: Nieuwenhuijsen MJ (ed) Exposure assessment in occupational and environmental epidemiology. University Press, Oxford, pp. 203–219
- Hong JH, Lee SI, Kim KE, Yong TS, Seo JT, Sohn MH, Shin DM (2004) German cockroach extract activates protease-activated receptor 2 in human airway epithelial cells. *J Allergy Clin Immunol* 113:315–319
- Houba R, Heederik DJJ, Doekes G, van Run PEM (1996) Exposure–sensitization relationship for α -amylase allergens in the baking industry. *Am J Respir Crit Care Med* 154:130–136
- International Union of Immunological Societies (IUIS). Allergen Nomenclature Sub-Committee (ed) List of allergens as of 7 June 2004. <http://www.allergen.org/list.htm>
- Johnsen CR, Sorensen TB, Larsen AI, Secher AB, Andreassen E, Kofoed GS, Nielsen LF, Gyntelberg F (1997) Allergy risk in an enzyme producing plant: a retrospective follow up study. *Occup Environ Med* 54:671–675
- Juniper CP, How MJ, Goodwin BF, Kinshott AK (1977) *Bacillus subtilis* enzymes: A 7-year clinical, epidemiological and immunological study of an industrial allergen. *J Soc Occup Med* 27:3–12
- Kämpe M, Stalenheim G, Edling C, Norbäck D, Almby B (1989) Enzyme allergies in research laboratories. *Clin Exp Allergy* 19:120
- Kizkin O, Suleyman GT, Hacıevliyagil S, Gunen H (2002) Proteolytic enzyme sensitivity and decrease in respiratory function (a 10-year follow-up). *Int Arch Occup Environ Health* 75:441–444
- Kurup VP, Xia JQ, Shen HD, Rickaby DA, Henderson JD Jr, Fink JN, Chou H, Kelly KJ, Dawson CA (2002) Alkaline serine proteinase from *Aspergillus fumigatus* has synergistic effects on Asp-f-2-induced immune response in mice. *Int Arch Allergy Immunol* 129:129–137
- Muir DC, Verrall AB, Julian JA, Millman JM, Beaudin MA, Dolovich J (1997) Occupational sensitization to lactase. *Am J Ind Med* 31:570–571

- Musk AW, Gandevia B (1976) Loss of pulmonary elastic recoil in workers formerly exposed to proteolytic enzyme (alcalase) in the detergent industry. *Br J Ind Med* 33:158–165
- Newhouse ML, Tagg B, Pocock SJ, McEwan AC (1970) An epidemiological study of workers producing enzyme washing powders. *Lancet* 1:689–693
- Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). Enzyme nomenclature. Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the nomenclature and classification of enzyme-catalysed reactions. <http://www.chem.qmul.ac.uk/iubmb/enzyme/>
- Palecek F, Palecekova M, Aviado DM (1967) Emphysema in immature rats. Condition produced by tracheal constriction and papain. *Arch Environ Health* 15:332–342
- Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE, Baur X (1998) Allergy to *Aspergillus*-derived enzymes in baking industry: identification of β -xylosidase from *Aspergillus niger* as new allergen (Asp n 14). *J Allergy Clin Immunol* 102:256–264
- Schweigert MK, Mackenzie DP, Sarlo K (2000) Occupational asthma and allergy associated with the use of enzymes in the detergent industry—a review of the epidemiology, toxicology and methods of prevention. *Clin Exp Allergy* 30:1511–1518
- Stewart GA, McWilliam AS (2001) Endogenous function and biological significance of aeroallergens: an update. *Curr Opin Allergy Clin Immunol* 1:95–103
- Stewart GA, Thomsen PJ, McWilliam AS (1993) Biochemical properties of aeroallergens: contributory factors in allergic sensitization. *Pediatr Allergy Immunol* 4:163–172
- Strassburger K, Bossert J, Baur X, Zober A (1999) Sensibilisierungen durch die Enzyme Phytase und Xylanase. In: Hallier E, Bünger J (eds) *Gesundheitsgefahren durch biologische Arbeitsstoffe*. 38. Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V. Rindt, Fulda, pp 525–527
- Vanhanen M, Tuomi T, Hokkanen H, Tupasela O, Tuomainen A, Holmberg PC, Leisola M, Nordman H (1996) Enzyme exposure and enzyme sensitisation in the baking industry. *Occup Environ Med* 53:670–676
- Vanhanen M, Tuomi T, Nordman H, Tupasela O, Holmberg PC, Miettinen M, Mutanen P, Leisola M (1997) Sensitization to industrial enzymes in enzyme research and production. *Scand J Work Environ Health* 23:385–391
- Weill H, Waddell LC, Ziskind M (1971) A study of workers exposed to detergent enzymes. *J Am Med Assoc* 217:425–433
- Wüthrich B, Ott F (1969) Berufsasthma durch Proteasen in der Waschmittelindustrie. *Schweiz Med Wochenschr* 99:1584–1586
- Zhu Z, Zheng T, Homer RH, Kim Y-K, Chen NY, Cohn L, Hamid Q, Elias (2004) Acidic mammalian chitinase in asthmatic Th1 inflammation and IL-13 pathway activation. *Science* 304:1678–1682