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Effect of High Pressure and Heat on Bacterial Toxins

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Summary

Even though the inactivation of microorganisms by high pressure treatment is a subject of intense investigations, the effect of high pressure on bacterial toxins has not been studied so far. In this study, the influence of combined pressure/temperature treatment (0.1 to 800 MPa and 5 to 121 °C) on bacterial enterotoxins was determined. Therefore, heat-stable enterotoxin (STa) of cholera toxin (CT) from *Vibrio cholerae*, staphylococcal enterotoxins A-E, haemolysin BL (HBL) from *Bacillus cereus*, and *Escherichia coli* (STa) were subjected to different treatment schemes. Structural alterations were monitored in enzyme immunoassays (EIAs). Cytotoxicity of the pressure treated supernatant of toxigenic *B. cereus* DSM 4384 was investigated with Vero cells. High pressure of 200 to 800 MPa at 5 °C leads to a slight increase of the reactivity of the STa of *E. coli*. However, reactivity decreased at 800 MPa and 80 °C to (66±21) % after 30 min and to (44±0.3) % after 128 min. At ambient pressure no decrease in EIA reactivity could be observed after 128 min. Pressurization (0.1 to 800 MPa) of heat stable monomeric staphylococcal toxins at 5 and 20 °C showed no effect. A combined heat (80 °C) and pressure (0.1 to 800 MPa) treatment lead to a decrease in the immuno-reactivity to 20 % of its maximum. For cholera toxin a significant loss in latex agglutination was observable only at 80 °C and 800 MPa for holding times higher than 20 min. Interestingly, the immuno-reactivity of *B. cereus* HBL toxin increased with the increase of pressure (182 % at 800 MPa, 30 °C), and high pressure showed only minor effects on cytotoxicity to Vero cells. Our results indicate that pressurization can increase inactivation observed by heat treatment, and combined treatments may be effective at lower temperatures and/or shorter incubation time.

Key words: high hydrostatic pressure, bacterial toxins, food safety

Introduction

The use of high hydrostatic pressure for the treatment of food in order to extend its shelf life and improve microbial safety is being developed because it offers the potential to alter the flavour and nutrient content of food to a lesser extent than conventional heat treatments. The effect of pressure treatment on the inactivation of microorganisms and protein denaturation is well documented. Pressure/temperature diagrams of several proteins have been published and their elliptic shape shows that there is an optimum temperature at which proteins are most resistant to pressure (*1*). While the dissociation of oligomeric proteins is supported by moderate pressures (<150 MPa), pressurization above 150–200 MPa induces unfolding of proteins. Beyond 200 MPa,

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significant tertiary structure changes are observed and at higher pressures (above 300–700 MPa) secondary structure changes take place, leading to non-reversible denaturation (*2*). However, the secondary structure of green fluorescent protein (27 kDa) is not influenced by pressures even up to 1300 MPa (*3*), indicating that protein structures may be very pressure resistant. Although the effect of pressure treatment on food relevant enzymes as peroxidase and polyphenol oxidase are available (*4,5*), the effect of high pressure on bacterial toxins has not been studied.

Enterotoxins of *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholerae* and pathovars of *Escherichia coli* are an important cause of a variety of diseases. Thermal stability of these proteins was used to classify them as heat-labile or heat-stable. However, the effect of combined pressure/temperature treatment on these differently heat-stable toxins is not known.

For more than 40 years, *B. cereus* has been recognized as a causative agent of food poisoning (*6*). It is known to cause two different types of food poisoning (*7*). The vomiting type of intoxication is caused by an emetic toxin produced by growing cells in the food (*8*). The emetic toxin, named cereulide, has a molecular mass of 1.2 kDa and remains active after heat treatment at 121 °C for 90 min, and stable at pH=2–11 (*9*). The diarrheal type is caused by various heat-labile enterotoxins. Treatment at 56 °C for 10 min leads to a complete loss of their biological activities (*10,11*). They are produced during vegetative growth of *B. cereus* in the small intestine (*7,8*). The most extensively studied enterotoxin is haemolysin BL (HBL), containing the protein components B (37.5 kDa), L_1 (38.2 kDa) and L_2 (43.5 kDa).

One of the main causes of food poisoning in North America is the ingestion of staphylococcal enterotoxins (SEs) produced by certain strains of *S. aureus* (*12*). Various SEs are recognized by the use of serological methods: A, B, C1, C2, C3, D, E, F, G, H, I and J (*9*). Heat stability is one of the most important properties of SEs in terms of food safety (*1*). Normal cooking times and temperatures are unlikely to completely inactivate the toxins SEA, SEB, and SEC (*13*). At 120 °C, the three toxins are completely inactivated in 20 to 30 min (*14*). Staphylococcal food-borne diseases are characterized by a short incubation period (2 to 6 h) after ingestion of preformed toxins, followed by nausea, vomiting, abdominal pain, and diarrhea (*1*). SEs are single polypeptides of approximately 25 to 28 kDa, have an overall ellipsoidal shape, and are folded into two unequal domains containing a mixture of α and β structures (*15*).

E. coli is a common commensal organism of the normal microflora in the intestinal tract of humans and warm–blooded animals. Most strains are non-pathogenic; however, some isolates, which were categorized by mechanisms of pathogenicity virulence properties, clinical syndromes, and distinct O:H serotypes, cause diarrhea. Enterotoxigenic *E. coli* (ETEC) strains colonize the surface of the small bowel mucosa and cause diarrhea through the action of two types of enterotoxins, heat-stable enterotoxins (STs), and heat-labile enterotoxins (LTs), whereby only ST, or LT, or both LT and ST may be expressed. STs are small, monomeric toxins that contain multiple cysteine residues, whose disulfide bonds account for the heat stability of these toxins. While STa is an 18- or 19-amino-acid peptide with a molecular mass of aproximately 2 kDa, the LTs of *E. coli* are oligomeric toxins of aproximately 86 kDa, composed of one 28 kDa A subunit and five identical 11.5 kDa B subunits that are closely related in structure and function to the cholera enterotoxin (CT) expressed by *V. cholerae* (*16*) and therefore have similar antigenic structures (*17*). CT is the toxin responsible for severe, choleralike disease in epidemic and sporadic forms. It is produced after vibrios have colonized the epithelium of the small intestine (*9*). Clinical disease is characterized by the passage of voluminous stools of rice water character that rapidly lead to dehydration (*18*).

It was the aim of this study to determine the effect of combined pressure/temperature treatment of enterotoxins from *S. aureus*, *B. cereus*, *V. cholerae* and *E. coli* (STa) on their reactivity in enzyme immunoassays (EIAs). Cytotoxicity of the pressure treated supernatant of toxigenic *B. cereus* DSM 4384 was investigated in order to compare its toxicity with the results obtained by the immunoassay.

Materials and Methods

Detection of the L2 components of the HBL complex

B. cereus DSM 4384 was grown at 32 °C in CYG medium (*19*) containing caseine 2 %, yeast 0.6 %, $(NH_4)_2SO_4$ 0.2 %, K_2HPO_4 1.4 %, KH_2PO_4 0.6 %, sodium citrate 0.1 % and $MgSO_4$ 0.2 % supplemented with glucose 1 % for 6 h. EDTA (1 mM) was added at the time of harvesting. Cell-free supernatants, obtained by centrifugation (10 000 × *g* at 4 °C for 20 min), followed by filtration through 0.2-μm-pore Millipore filters, were used in the enzyme immunoassay (EIA). For the determination of L2 component of the HBL in cell-free supernatants, the microtiter plates were coated with serial dilutions of the supernatants. The enzyme immunoassay, based on monoclonal antibodies, was performed according to Dietrich *et al.* (*20*). The antibodies 1A12 and 8B12 were specific for the L2 component. Free protein binding sites of the plates were blocked with phosphate-buffered saline containing sodium caseinate (30 g/L) for 30 min. Subsequently, 100 μL of the respective purified monoclonal antibody (2 μg/mL) were added, and the plates were developed as described in Dietrich *et al.* (*20*). Data are presented as means of three independent experiments and error bars indicate standard deviation. An untreated sample was used as a control, representing 100 % reactivity, and a dilution of 1:320 gave absorbance values at 450 nm of 1.12, 0.96, and 0.94, respectively.

Determination of the cytotoxicity

Cytotoxicity of the cell-free supernatants was determined by measuring cell proliferation and cell viability using Vero cells (*20*). Growth medium and diluent consisted of Eagle minimum essential medium (Biochrom KG, Berlin, Germany) with Earle salts supplemented with 1 % calf serum and glutamine 2 mM. The activity was tested as serial dilutions in microtiter plates. Cell-free supernatant (0.1 mL) was added to 0.1 mL of the Vero cells, and the plates were incubated for 24 h at 37 °C in a CO_2 atmosphere 5 %. Cell proliferation reagent WST-1

(10 μL) (Roche Diagnostics, Mannheim, Germany) was added to 0.1 mL of the above suspension, and the plates were incubated for another hour under the same conditions. The absorbance was determined at 450 nm, and the 50 % inhibitory concentration was calculated as described by Dietrich *et al.* (*20*). Data are presented as means of three independent experiments and error bars indicate standard deviation. An untreated sample was used as control, representing 100 % cytotoxicity, and the dilution that gave a 50 % reduction in the survival rate of the Vero cells was 1:348, 1:575, and 1:758, respectively.

Detection of the staphylococcal enterotoxins

RIDASCREEN® EIA kits, which utilize five monovalent capture antibodies against SEA to SEE, and a mixture of SET A, B, C, D, and E with a concentration of 2 ng/mL for each toxin, were obtained from R-Biopharm GmbH, Darmstadt, Germany. The toxin mixture was diluted with phosphate buffered saline – PBS ($NaH_2PO_4 \cdot H_2O$ 0.55, $Na_2HPO_4 \cdot 2\ H_2O$ 2.85 and NaCl 8.7 (in g/L), and the pH was adjusted to 7.4) to a concentration of 1.4 ng/mL, transferred to 0.5-mL Eppendorf reaction tubes, sealed with silicon stoppers avoiding enclosure of air, and stored on ice until pressure treatment. Afterwards, 400 μL of each sample were diluted with 300 μL of PBS. The enterotoxin assays were performed by the methods recommended by the manufacturers of the kits. Coloured extracts resulting from the enzymatic reactions were measured by determining absorbance at 450 nm with a Spectraflour microtiter plate reader (Tecan, Grödig, Austria). Data are presented as means of two independent experiments and error bars indicate standard deviation. An untreated sample was used as control, representing 100 % reactivity, and gave absorbance values at 450 nm of 1.036 and 1.061 for SEC.

Detection of the heat-stable E. coli enterotoxin

Test kits for the detection of heat-stable *E. coli* enterotoxin by competitive enzyme immunoassay, and *E. coli* heat-stable enterotoxin STa were obtained from Oxoid GmbH, Wesel, Germany. Based on the competitive EIA format, the test uses a synthetic peptide toxin analogue and a monoclonal antibody to ensure specificity. A mass of toxin (10 μg) was diluted with 50 mL of TE buffer (10 mM TRIS-HCl, 1 mM EDTA, and the pH was adjusted to 8), transferred to 0.5-mL Eppendorf reaction tubes, sealed with silicon stoppers avoiding enclosure of air, and stored on ice until pressure treatment. Enterotoxin assays were performed by the methods recommended by the manufacturers of the kits. An untreated sample was used as control, representing 100 % reactivity, and gave absorbance values at 485 nm of 0.56±0.1. TE buffer without the added toxin as assay blank showed values of 1.38±0.23. The A_{485nm} of the samples was subtracted from that of TE buffer without the added toxin, multiplied by –1 and related to the untreated sample. Data are presented as means of two independent experiments and error bars indicate standard deviations.

Detection of Vibrio cholerae enterotoxin

Test kits for the detection of cholera toxin from *Vibrio cholerae* by reversed latex agglutination, which utilize polyvalent antibodies, and cholera toxin from *Vibrio cholerae* were obtained from Oxoid GmbH, Wesel, Germany. A mass of toxin (500 μg) was diluted with 150 mL of TE buffer, transferred to 2-mL Eppendorf reaction tubes, sealed with silicon stoppers avoiding enclosure of air, and stored on ice until pressure treatment. Enterotoxin assays were performed and classified by the methods recommended by the manufacturers of the kits.

Pressure treatment

The samples were pressurized at temperatures ranging from 5 to 80 °C and pressures ranging from 0.1 to 800 MPa using a FoodMicroLab equipment (Stansted Fluid Power Inc., Stansted, UK). The compression and decompression rates were 2 MPa/s, the temperature of the pressure cell was maintained by a water bath and monitored by a thermocouple in the autoclave. The internal volume of pressure vessels was 30 mL and ethanol-castor oil (80:20) was used as pressure transmission fluid. The temperature in the samples rose by approximately 20 °C due to adiabatic heating and the temperature profile for treatments at 80 °C and 600 or 800 MPa are shown in Table 1. Comparable temperature profiles were obtained for treatments with 400 MPa (data not shown). After decompression, the sample tubes were stored on ice until immunological or cytotoxical determination.

Table 1. Pressure-temperature profiles for treatments in buffer at 600 and 800 MPa and 80 °C

p=600 MPa			*p*=800 MPa		
t/min*	*p*/MPa	*T*/°C	*t*/min*	*p*/MPa	*T*/°C
–5.0	0.10	79.9	–6.7	0	79.3
–2.5	208	93.9	–4.2	173	91.7
–0.5	439	98.9	–2.2	403	98.8
0.3	604	101.7	–1.0	512	98.6
1.0	597	99.8	–0.2	621	97.9
2.0	592	94.0	0.6	816	101.0
3.1	589	89.3	1.3	809	98.4
4.1	588	86.3	2.4	804	92.9
6.1	588	83.3	4.3	801	86.2
8.0	587	82.1	6.3	800	83.2
11.0	588	80.1	9.3	800	81.6

*0 min is defined as the time when the maximum pressure level is reached

Statistical treatment

All data shown are means of at least two independent experiments, and error bars indicate standard deviation.

Results

Effect of pressurization on the reactivity of heat-stable enterotoxin STa of E. coli in the EIA

Data were obtained of the smallest of the tested toxins after the combined pressure/temperature treatment for 30 min in the range of 0.1 to 800 MPa at 5 and 80 °C.

Pressurization from 200 to 800 MPa at 5 °C leads to a slight increase of the reactivity. However, reactivity decreased to (66±21) % at 800 MPa and 80 °C (Fig. 1). Furthermore, the reactivity of STa in the EIA was monitored at 80 °C at 0.1 and 800 MPa over a period of 128 min (Fig. 2). At ambient pressure no decrease in EIA reactivity could be observed even after 128 min. Likewise, treatment at 121 °C for 30 min showed no effect (data not shown). In contrast, reactivity decreased at 800 MPa and 80 °C to (44 ±0.3) % after 128 min of pressure holding time.

Reactivity of pressurized staphylococcal enterotoxins in EIAs

One of the most important properties of SEs in terms of food safety is their heat stability. To examine whether these relatively small toxins also exhibit high pressure stability, the effect of combined pressure/temperature treatment on the reactivity of SEA to SEE in the EIA after 30 min at 5 and 20 °C, and after 30 and 120 min at 80 °C was determined. The results for SEC are shown in Fig 3. Pressurization at 5 and 20 °C in the range of 0.1 to 800 MPa showed no effect (Fig 3., and data not shown). At ambient pressure EIA reactivity of SEC decreased by 35 % after 30 min and by 63 % after 120 min at 80 °C. Pressure treatment at 80 °C for 30 min in the range of 0.1 to 400 MPa showed a slight increase and from 400 to 800 MPa again a slight decrease in the immuno-reactivity. Pressurization for 120 min at 80 °C had almost no additional effect. Only after 120 min at 800 MPa and 80 °C pressurization leads to a further decrease in reactivity. The effect of pressure on SEA to SEE did not differ (data not shown). However, thermal stability varied strongly. The order of heat resistance at 80 °C was SEA=SEC=SEE>SED>SEB (data not shown).

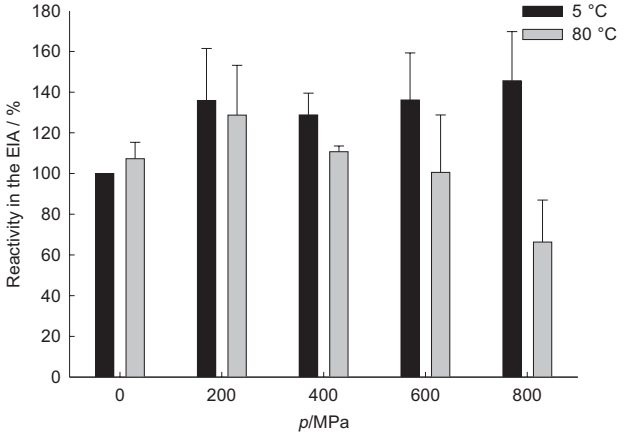

Fig. 1. Effect of pressurization on the reactivity of heat-stable enterotoxin STa of *E. coli* in the EIA after 30 min at 5 and 80 °C. Data shown are means of two independent experiments and error bars indicate standard deviation. An untreated sample was used as control, representing 100 % reactivity, and gave absorbance values at 485 nm of 0.56±0.1. TE buffer as assay blank showed values of 1.38±0.23

Effect of pressure on the detection of cholera toxin by reversed passive latex agglutination

The multimeric CT was subjected to combined pressure/temperature treatment for 30 min in the range of 5 to 121 °C and 0.1 to 800 MPa. At 5, 40, and 60 °C and a pressure level from 0.1 to 800 MPa all samples could be classified at a titer of 1:128 as positive (+++), so that no difference of the agglutination pattern to the untreated sample could be observed (data not shown). At 80 °C the detectable toxin concentration slightly decreased, leading at 800 MPa to a negative reaction (±) at a titer of 1:128 (Table 2). Therefore, kinetics were determined. Although CT was still detectable after a period of 90 min at 800 MPa and 80 °C, toxin concentration decreased close to the detection limit (Table 3). Incubation at 80 °C and 0.1 MPa for the same period caused no reduction. However, after 30 min at 121 °C the concentration of the detectable cholera toxin decreased under the detection limit.

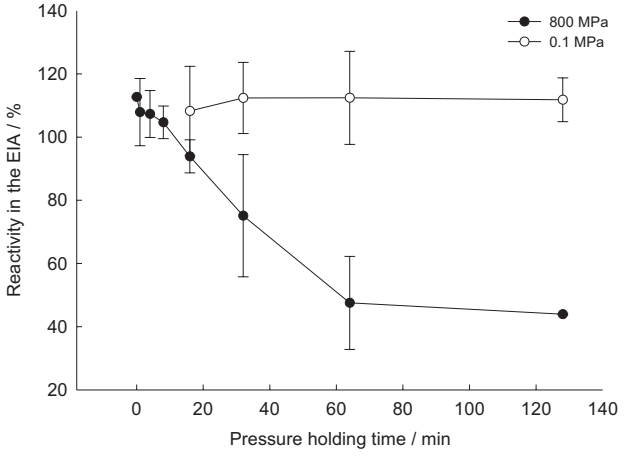

Fig. 2. Effect of pressurization on the reactivity of heat-stable enterotoxin STa of *E. coli* in the EIA at 80 °C. Data shown are means of two independent experiments and error bars indicate standard deviation. An untreated sample was used as control, representing 100 % reactivity, and gave absorbance values at 485 nm of 0.56±0.1. TE buffer as assay blank showed values of 1.38±0.23

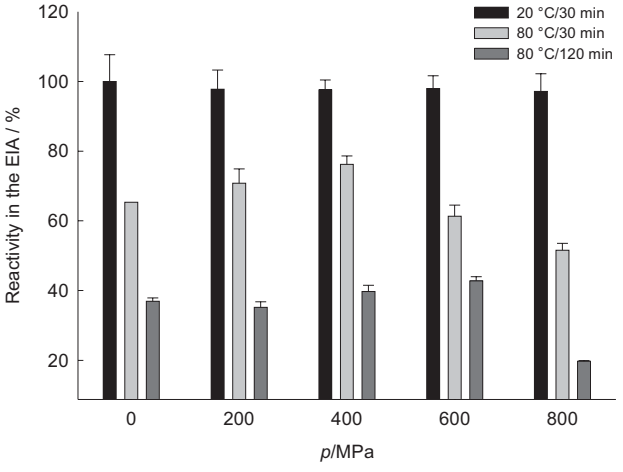

Fig. 3. Effect of pressurization on the reactivity of staphylococcal enterotoxin C in the EIA after 30 min at 20 °C, and after 30 and 120 min at 80 °C. Data shown are means of two independent experiments and error bars indicate standard deviation. An untreated sample was used as control, representing 100 % reactivity

Table 2. Detection of *Vibrio cholerae* enterotoxin after combined pressure/temperature treatment for 30 min*

LT/ titer	*p*/MPa					
	T=80 °C					*T*=121 °C
	0.1	200	400	600	800	0.1
1:2	+++	+++	+++	+++	+++	±
1:4	+++	+++	+++	+++	+++	–
1:8	+++	+++	+++	+++	+++	–
1:16	+++	+++	+++	+++	+++	–
1:32	+++	+++	+++	+++	++	–
1:64	+++	+++	+++	++	+	–
1:128	+++	++	++	++	±	–

*Interpretation of the test results was performed by the methods recommended by the manufacturers of the kit. Results classified as (+), (++) and (+++) are considered to be positive

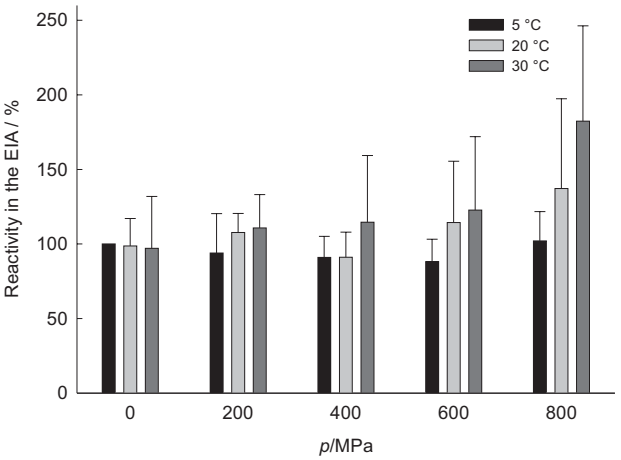

Fig. 4. Effect of pressurization at 5, 20 and 30 °C on the reactivity of the supernatant of *B. cereus* DSM 4384 in the EIA after 30 min of pressure holding time. Data shown are means of three independent experiments and error bars indicate standard deviation. An untreated sample was used as control, representing 100 % reactivity

Reactivity in EIA and cytotoxicity of the supernatant of toxigenic B. cereus after pressure treatment

The diarrheal type of intoxication of *B. cereus* is caused by multimeric enterotoxins that are characterized as heat-labile. In order to determine the pressure resistance of the largest toxin of our study, the effect of pressure treatment on the reactivity in the EIA of the supernatant of *B. cereus* DSM 4384 was investigated (Fig. 4). EIA reactivity was slightly enhanced as pressure increased. Thereby, the pressure-induced increase was more pronounced at higher temperatures, leading to a maximum reactivity of (182±63) %. Additionally, we determined the cytotoxicity of the samples as a measure of biological activity (Fig. 5). Likewise, pressure treatment had almost no effect on *B. cereus* enterotoxins, but in contrast to the results obtained by the EIA, an increase of the pressure level in the range of 0.1 to 800 MPa at 5 °C resulted in a slight decrease of the toxicity to 81 % at maximum pressure. Even if the temperature was increased up to 30 °C at the same pressure level, no further significant decrease of the toxicity could be observed.

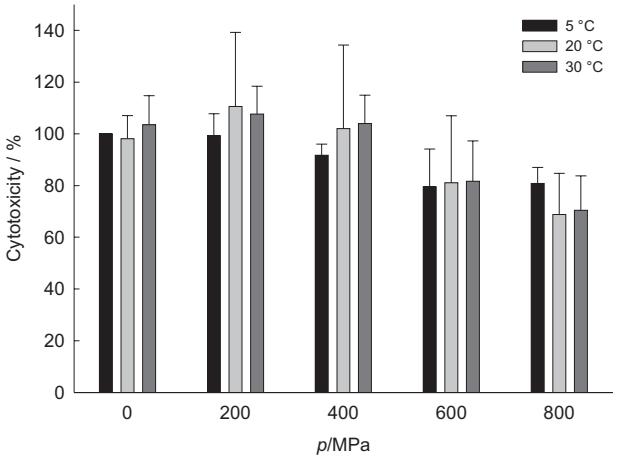

Fig. 5. Effect of pressurization at 5, 20 and 30 °C on the cytotoxicity of the supernatant of *B. cereus* DSM 4384 after 30 min of pressure holding time. Data shown are means of three independent experiments and error bars indicate standard deviation. An untreated sample was used as control, representing 100 % cytotoxicity, and the dilution that gave a 50 % reduction in the survival rate of the Vero cells was 1:348, 1:575, and 1:758, respectively

Table 3. Detection of *Vibrio cholerae* enterotoxin after combined pressure/temperature treatment at 80 °C*

LT/titer	Pressure holding time/min											
	p=800 MPa									*p*=0.1 MPa		
	10	20	30	40	50	60	70	80	90	30	60	90
1:2	+++	+++	+++	+++	+++	++	++	++	+	+++	+++	+++
1:4	+++	+++	+++	+++	+++	++	++	+	±	+++	+++	+++
1:8	+++	+++	+++	+++	++	+	+	±	–	+++	+++	+++
1:16	+++	+++	+++	+++	++	+	+	±	–	+++	+++	+++
1:32	+++	+++	++	++	+	±	±	–	–	+++	+++	+++
1:64	+++	++	+	+	±	–	–	–	–	+++	+++	+++
1:128	++	+	±	±	–	–	–	–	–	+++	++	++

*Interpretation of the test results was performed by the methods recommended by the manufacturers of the kit. Results classified as (+), (++) and (+++) are considered to be positive

Discussion

As a thermodynamic parameter, pressure has been known for many years to act on biological materials in a different way from temperature (*2*).

The four bacterial enterotoxins chosen show different structures and also resemble different biological activities. SEs are single polypeptides of approximately 25 to 28 kDa (*15*). The enterotoxins of *B. cereus*, *V. cholerae* and *E. coli* are mainly produced in the intestine after the ingestion of food contaminated with the respective bacteria, but if the bacteria have grown to high numbers in the food, production of toxins cannot be excluded. The main reason for the choice of these toxins was that they resemble different secondary structures (see introduction), so one could expect differing effects of high pressure with or without heat treatment.

Summarizing the effects observed on immuno-reactivity in the respective assays, there was no effect of pressurization of up to 800 MPa at ambient (20 °C) or lower (5 °C) temperatures. Some reduction of immuno--reactivity could be demonstrated when high pressure treatment was combined with a heating step. Particularly for STa (1.2 kDa), a significantly reduced EIA result was obtained using 80 °C and a pressure of 800 MPa, while incubation at 121 °C for 30 min or at 80 °C for 124 min at ambient pressure showed no effect. In contrast, comparatively small effects of pressure and strong effects of heat treatment on SEC (25–28 kDa) could be observed. The different behaviour of the two monomeric proteins indicates that heat and pressure resistance of bacterial toxins do not correlate. Likewise, no correlation was found for the resistance of vegetative cells (*21,22*) and bacterial endospores to pressure and heat, respectively (*23–25*). Furthermore, SEC was stabilized at 80 °C in the middle pressure range. Pressure/temperature diagrams of other proteins also show that there is an optimum pressure at which proteins are most resistant to heat treatment (*1*).

In a similar way as the monomeric STa, the multimeric cholera toxin (86 kDa) became negative in the RPLA after a combined pressure (800 MPa) and heat (80 °C) treatment for 90 min, whereas this was not observed without pressurization. The additive effect of the temperature rise due to adiabatic heating can be disregarded, as the starting temperature is reached again after a pressure holding time of 11 min (Table 1). As expected (see introduction), heat resistance at 121 °C of STa and CT differed strongly. The concentration of the detectable CT decreased under the detection limit after 30 min. The observed relative high resistance of CT in the RPLA to both, heat and/or pressure treatment, could be explained by the use of polyclonal antibodies.

It can be generally assumed that the loss of immuno-reactivity is mainly due to changes in tertiary structure and that the biological activity should also decrease. Particularly, studies on heat inactivation of SEs widely used the assumption that loss of reaction with the specific antibodies indicates inactivation (*26*). To verify this thesis, the results of an immunoassay of the pressure treated supernatant of toxigenic *B. cereus* were compared to its cytotoxicity. The results of both methods showed that pressurization in the range of 0.1 to 800 MPa at 5, 20 and 30 °C has almost no effect even on the largest toxin of our study (119 kDa). There was, however, a slight increase of the immunoassay result for the L2 component of the HBL enterotoxin complex at 20 and 30 °C and a pressure of 800 MPa, whereas the respective cytotoxicity decreased. One explanation for this observation could be that increasing pressure leads to a dissociation of the HBL complex and therefore to a better accessibility of the L2 molecules for the antibodies, which might partly be hidden in the test mixture used for this study under normal conditions. A similar effect is known as antigen retrieval in immunohistochemistry where antibody accessibility is induced *e.g.* by heat (*27*). Oligomeric proteins such as glyceraldehydes-3-phosphate dehydrogenase (GAPDH) from yeast, malate dehydrogenase, and lactate dehydrogenase (LDH) were also found to dissociate through pressure treatment (*28–31*).

Overall these results indicate that pressure application may increase inactivation by heat treatment and combined treatments may be effective at lower temperatures and/or shorter incubation time. Furthermore, it must be emphasized that no epitope masking of bacterial toxins was observed after pressurization, which admits their detection after pressure based food processing.

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Utjecaj visokoga tlaka i topline na bakterijske toksine

Sažetak

Iako se intenzivno ispituje inaktivacija mikroorganizama primjenom visokoga tlaka, do sada nije proučen njegov utjecaj na toksine bakterija. U radu je utvrđen zajednički utjecaj tlaka i temperature (0,1–800 MPa i 5–121 °C) na bakterijske enterotoksine. Stoga su toplinski stabilni enterotoksin iz *Escherichia coli* (STa), toksin kolere (CT) iz *Vibrio cholerae*, enterotoksini stafilokoka A–E i hemolizin BL (HBL) iz *Bacillus cereus* bili podvrgnuti različitim uvjetima obrade tlakom i temperaturom. Strukturne promjene ispitivane su enzimskim imunoesejima. Citotoksičnost, pod tlakom obrađenog *B. cereus* DSM 4384, ispitana je s Vero stanicama. Visoki tlak od 200 do 800 MPA pri 5 °C uzrokuje blagi porast reaktivnosti STa iz *E. coli*, ali se pri 800 MPa i 80 °C tijekom 30 minuta smanjuje reaktivnost do (66±21) %, a nakon 128 min do (44±0,3) %. Pri atmosferskom tlaku nije opaženo smanjenje reaktivnosti ni nakon 128 min. Tlak od 0,1 do 800 MPa nije imao učinka na toplinski stabilne monomerne toksine stafilokoka pri 5 i 20 °C. Kombiniranim utjecajem temperature i tlaka (80 °C i 0,1–800 MPa) smanjena je imunoreaktivnost na 20 %. Značajno smanjenje aglutinacije lateksa toksinom kolere opaženo je samo pri 80 °C i 800 MPa ako je postupak trajao dulje od 20 minuta. Zanimljivo je da se imunoreaktivnost HBL toksina iz *B. cereus* povećavala s povisivanjem tlaka (182 % pri 800 MPa i 30 °C), a visoki je tlak samo malo utjecao na citotoksičnost *B. cereus* na Vero stanice. Iz ovih se rezultata vidi da povišeni tlak povećava inaktivaciju koju opažamo pri toplinskoj obradi, a zajedničkom primjenom tlaka i temperature postižu se dobri rezultati pri nižim temperaturama i/ili kraćem vremenu inaktivacije.