The *in situ* observation of the temperature and pressure stability of recombinant *Aspergillus aculeatus* pectin methylesterase with Fourier transform IR spectroscopy reveals an unusual pressure stability of β-helices

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The stability of recombinant *Aspergillus aculeatus* PME (pectin methylesterase), an enzyme with high β-helix content, was studied as a function of pressure and temperature. The conformational stability was monitored using FTIR (Fourier transform IR) spectroscopy whereas the functional enzyme stability was monitored by inactivation studies. Protein unfolding followed by amorphous aggregation, which makes the process irreversible, was observed at temperatures above 50 °C. This could be correlated to the irreversible enzyme inactivation observed at that temperature. Hydrostatic pressure greater than 1 GPa was necessary to induce changes in the enzyme's secondary structure. No enzyme inactivation was observed at up to 700 MPa. Pressure increased PME stability towards thermal denaturation. At 200 MPa, temperatures above 60 °C were necessary to cause significant PME unfolding and loss of activity. These results may be relevant for an understanding of the extreme stability of amyloid fibrils for which β-helices have been proposed as a structural element.

Key words: amyloid, β-helix, enzyme inactivation, Fourier transform IR spectroscopy (FTIR), hydrostatic pressure, pectin methylesterase (PME).

INTRODUCTION

Knowledge regarding the stability of the enzyme, as a function of processing conditions, is mandatory to apply exogenous enzymes in an intelligent and efficient manner. PME (pectin methylesterase, EC 3.1.1.11) catalyses the de-esterification of pectin, a polysaccharide occurring in the middle lamellae of plant cell walls. PME is used for various applications in fruit processing e.g. texture improvement of fruit pieces [1–3], juice extraction, concentration and clarification of fruit juices [4,5]. Most of the exogenous PME used in food processing is obtained from fungal sources, mainly *Aspergillus* species [6]. PMEs show some interesting structural features, the most prominent being the presence of β-helices [7]. Since β-helices have been proposed as a structural element of amyloid fibrils [7,8] it is interesting to correlate the properties of these protein structures. It has been demonstrated previously that recombinant *Aspergillus aculeatus* PME can be inactivated by high temperatures (46–56 °C) but is hardly inactivated by pressure treatments up to 700 MPa even in combination with elevated temperature (55 °C) [9]. Recombinant *Aspergillus aculeatus* PME is the only fungal PME for which data regarding temperature and/or pressure stability are available in open literature. Similarly, the extreme pressure stability of mature amyloid fibrils has recently been demonstrated [10].

A crystal structure for *Aspergillus aculeatus* PME, or for any other fungal PME, has not yet been determined, only its amino acid sequence [11]. So far, X-ray diffraction studies have elucidated the crystal structure for bacterial PME from *Erwinia chrysanthemi* [12] and plant PME from carrot [13]. A computer-generated 3D-model of tomato PME was also constructed [14]. All three enzymes were proposed to fold into a right-handed parallel β-helix, an architecture similar to the one reported for other pectinolytic enzymes [7,15], with a β-helix as the predominant structural unit. The right-handed parallel β-helix was proposed as the general structure for plant PME [13], shown in Figure 1 [16]. However, after comparison with the amino-acid sequences of 127 PMEs and calculation of an evolutionary tree for 70 representative enzymes of this family, Markovic and Janecek [17] suggested that based on the significant sequence differences between plant, fungal and bacterial PME, conclusions based on the 3D structures of *E. chrysanthemi* and carrot PME could be valid only for particular clades or specific groups of PME. FTIR (Fourier transform IR spectroscopy) enables us to determine the amount of each secondary structure present in a protein and so to validate that the PME under study consists mainly of a β-structure, as this is the main component present in the β-helix.

Enzyme inactivation can be due to a small change in the active site, but is usually coupled to a global conformational change in the protein. An alternative method to investigate enzyme stability is therefore to monitor modifications of the protein conformation during processing *in situ*. These conformational changes can be observed using FTIR, which provides information on the secondary structure of the enzyme. IR absorptions for the peptide bonds of a protein give rise to several so-called amide bands, amide I' being the most important one among them. This band between 1600 and 1700 cm^{-1} is composed of various overlapping subcomponent bands. These individual bands can each be assigned to a specific secondary structural element (β-structures, α-helix, turn-and-bends, and unordered structures). Mathematical techniques for band narrowing such as Fourier deconvolution can be applied to analyse a complex signal and to identify individual bands indicative of different secondary structure components.

Abbreviations used: BPTI, bovine pancreatic trypsin inhibitor; DAC, diamond anvil cell; FTIR, Fourier transform IR spectroscopy; HRP, horseradish peroxidase; PME, pectin methylesterase.

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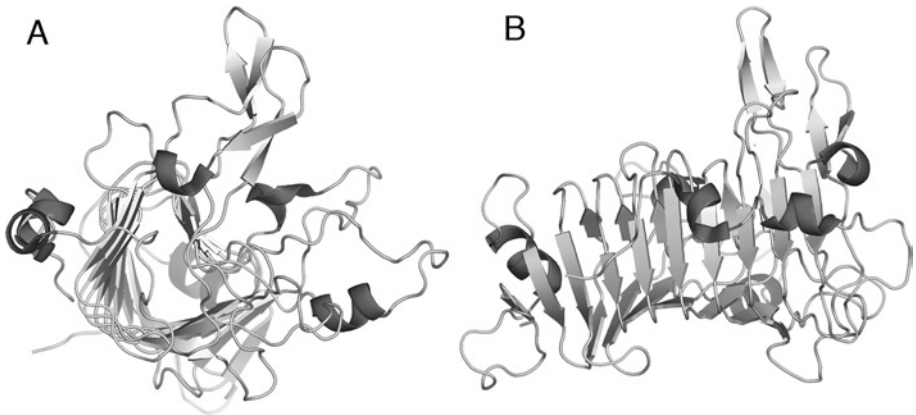

Figure 1 Schematic representation of PME from *Erwinia chrysanthemi* species, prepared with MOLSCRIPT [16]

One can see that the predominant structures are 3 parallel β-sheets, comprising the β-helix. Apart from that, some more or less regular α-helices, a 4-stranded anti-parallel β-sheet and turn-structures connecting the strands can be found: (**A**) sideview; (**B**) topview. PDB-code: 1QJV.

The aim of this study is to explore the stability of recombinant *Aspergillus aculeatus* PME at elevated temperatures and pressures by inactivation experiments. FTIR spectroscopy in a temperature-regulated DAC (diamond anvil cell) additionally offers the opportunity to correlate functional enzyme stability to the conformational stability of PME during temperature and pressure treatment. Moreover, FTIR measurements in the DAC allow pressure treatments to reach greater than 1 GPa, higher than most other techniques offer. Temperature-induced inactivation and unfolding of PME are correlated. Both inactivation and unfolding of the enzyme were shifted to a higher temperature at moderate pressure (200 MPa). At room temperature a change in the conformation of PME was only observed above 1 GPa.

EXPERIMENTAL

Sample preparation

A commercial preparation of recombinant *Aspergillus aculeatus* PME (Novoshape, Novozymes, Denmark) was used as a PME source. This preparation is generally recommended as safe (GRAS) for application in food processing [18]. The commercial preparation was dialysed against an excess of demineralized water for 48 h with regular changing of the water to remove any salt, the absorption of which could interfere with the amide I' region. This dialysed solution was subsequently freeze-dried.

For the inactivation experiments freeze-dried PME was dissolved in sodium-acetate buffer 0.1 M (pH 4.5) to a concentration of 0.05 mg/ml suitable for activity measurement. For the FTIR experiments the freeze-dried PME was dissolved in D_2O to a concentration of 20 mg/ml and left overnight to ensure that all accessible protons were exchanged for deuterons. The final pD of the solution was 5. The pH meter was calibrated by reference to standard buffers in H_2O and corrected for the deuterium isotope effect [19] (pD = pH meter reading + 0.4). The sample was centrifuged just before measurement to precipitate all aggregates that were potentially formed.

PME activity assay

PME activity was determined by a pH stat titration (Titrino, Metrohm) of the acid produced with a mixture consisting of 250 μl PME and 30 ml of 3.5 mg/ml apple pectin (70–75 % esterification, Fluka) solution containing 0.117 M NaCl, pH 4.5 at 22 °C. The PME activity unit is defined as the amount of enzyme required to release 1 μmol of acid per minute, under the above mentioned assay conditions.

Inactivation study: isothermal–isobaric treatments

The inactivation of recombinant *Aspergillus aculeatus* PME was studied within the temperature range 20–65 °C at 0.1 MPa, 200 MPa, 400 MPa and 700 MPa (the highest pressure feasible using available equipment). Isothermal treatments at atmospheric pressure were performed in a temperature-controlled water bath, with the enzyme solution enclosed in capillary tubes. After 15 min, the samples were withdrawn from the water bath and immediately cooled in ice-water. The residual PME activity was measured within 1 h of storage. During storage in ice-water no reactivation of the enzyme was observed. Isobaric–isothermal treatments were conducted in laboratory scale high-pressure equipment (Resato, Roden, The Netherlands) as described in Weemaes et al. [20]. The enzyme samples, in 0.25 ml flexible microtubes, were enclosed in the pressure vessels that were previously equilibrated to a certain temperature. Pressure was raised slowly (100 MPa/min) to minimize temperature increases due to adiabatic heating. After pressure build-up an equilibration period of 2 min was taken into account to allow temperature to evolve to its desired value. At that moment, the experimental time was started. At 0 and 15 min vessels were individually depressurized and samples were immediately cooled in ice-water. The residual PME activity was measured within 1 h of storage time. During storage in ice-water no reactivation of PME was observed.

FTIR spectroscopy

Temperature scans at atmospheric pressure were performed in a cell containing CaF_2 windows separated by 50 μm Teflon spacers. The cell was placed in a heating jacket and the temperature was increased automatically by a Graseby Specac temperature controller at a rate of 1 °C/5 min. Pressure scans and temperature scans at elevated pressure were performed in a DAC (Diacell Products, Leicester, U.K.). A droplet of the solution and some barium sulphate were deposited on a stainless steel gasket, which was positioned in between the two diamonds. The DAC was then placed in the minicell, which was allowed to manually build up the pressure. Pressure was determined by monitoring

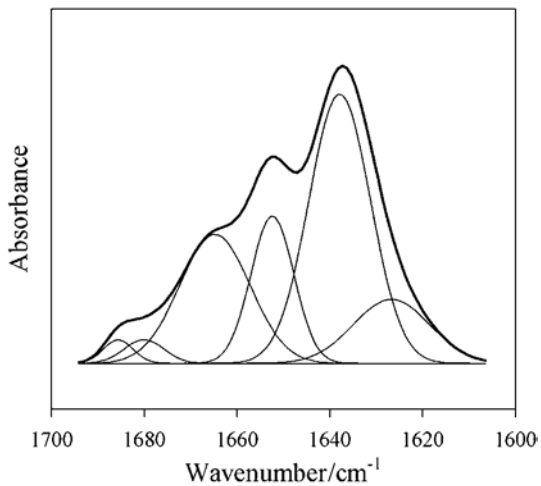

Figure 2 Deconvoluted amide I' band of PME at atmospheric pressure and room temperature

The predominant structure in PME *A. aculeatus* is the β-helix, as can be deduced from the band at 1638 cm^{-1}. Other structures present are α-helices and some turns-and-bends. The exact assignment of each band can be found in Table 1.

Table 1 Secondary structure elements of native PME deduced from curve-fitting to the deconvoluted amide I' band

Wavenumber (cm^{-1})	Area (%)	Structural unit
1627	13	β-sheet or extended chain
1638	43	β-helix
1652	16	α-helix
1665	24	Turns-and-bends
1680	2	Turns-and-bends
1686	2	Turns-and-bends

the pressure-dependent absorption of the sulphate stretching of barium sulphate. For pressure scans, the DAC was maintained at 25 °C. The IR spectra were taken using a Bruker IFS66 (Karlsruhe, Germany) FTIR spectrometer equipped with a liquid-nitrogen-cooled mercury-cadmium-telluride detector. Interferograms (256) were taken at a resolution of 2 cm^{-1} and co-added to obtain a good signal-to-noise ratio. Dry air was constantly purged through the spectrometer. Fourier deconvolution was performed using Bruker software. The assumed line shape was Lorentzian. A half bandwidth of 21 cm^{-1} and an enhancement factor of 1.7 were used. Curve-fitting which permits a quantitative analysis of the amide I' band was carried out using a home-made program, Proteir [21].

RESULTS AND DISCUSSION

Secondary structure components of native PME

The deconvoluted amide I' band of PME at atmospheric pressure and room temperature is shown in Figure 2. The contribution of all the secondary structures present can be calculated from the integrated areas under each band. The position of these bands can be assigned to a specific type of secondary structure [22,23]. A major band at 1638 cm^{-1} is observed, which can be attributed to β-helix structure, as a band at this position has already been noted in other β-helical proteins [24]. Table 1 summarizes the secondary structure elements of native PME deduced from curve-fitting of the deconvoluted amide I' band. The main structure comprising recombinant *Aspergillus aculeatus* PME is clearly β-helix. Other

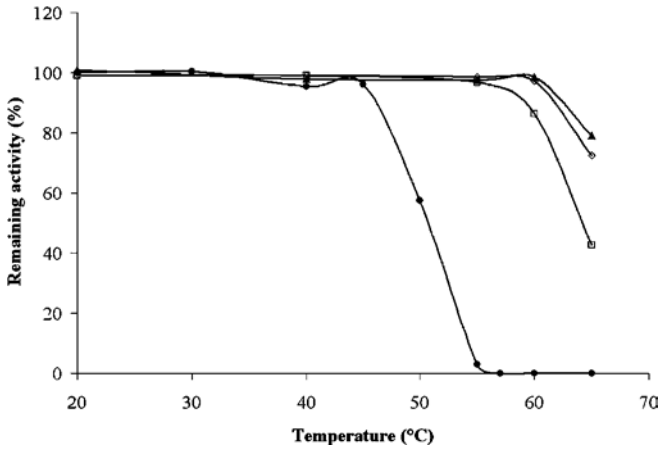

Figure 3 Remaining PME activity after a 15 min treatment at different temperatures at 0.1 MPa (●), 200 MPa (□), 400 MPa (▲), 700 MPa (◇)

Although inactivation at atmospheric pressure already begins at temperatures above 45 °C, no inactivation can be detected after a 15 min treatment at 55 °C at higher pressures (200, 400 and 700 MPa). Higher temperatures are then required to obtain a certain degree of inactivation, at 400 MPa for example a 15 min treatment at 65 °C causes only 21 % of inactivation.

structures present are α-helices and some turns and bends. The assignment of the band at 1627 cm^{-1} is more ambiguous since this band can arise from a β-sheet structure or extended chain. Because PME, both from carrot (*Daucus carota*) [17] and *Erwinia chrysanthemi* [12], has a small anti-parallel β-sheet (although in another conformation) and an extended chain at the C-terminus (although of different length) both options or a combination are possible.

Although there can be differences both between the loops extruding from the β-helix and at its terminus (as has been shown by the different crystal structures) the assumption that all pectinases have a right-handed parallel β-helix as the main structural element is supported by the FTIR results. Findings indicate that the β-helix is the predominant structural unit of recombinant *Aspergillus aculeatus* PME, present in approximately the same amount as in tomato PME [14]. On the basis of CD data, it is estimated that the percentage of β-structure present in the enzyme is 47 %. Our own calculations, based on the available X-ray diffraction data indicated that plant PME has a slightly higher content of β-helical structure than PME from microbial origin (51 % in β-helix for carrot and 45 % in *Erwinia chrysanthemi*, which has more loops extruding from the β-helix). Since *Aspergillus aculeatus* is more closely related to *Erwinia chrysanthemi* from an evolutionary point of view [17], the structural data obtained for *Aspergillus aculeatus* PME using FTIR are in good accordance with these results.

Effect of temperature treatment at atmospheric pressure on PME stability

The PME activity remaining after 15 min treatment at different constant-temperature–pressure conditions was assayed (Figure 3). At atmospheric pressure PME is inactivated at temperatures higher than 45 °C. After a treatment of 15 min at 50 °C 57 % of the initial activity is retained, whereas only 3 % of the activity remains after 15 min at 55 °C. These results are in agreement with data from the kinetic inactivation study of recombinant *Aspergillus aculeatus* PME in the temperature range 50–55 °C, reported previously [9]. In order to determine whether thermal inactivation is correlated with a global conformational change to the enzyme, the protein's secondary structure was monitored by

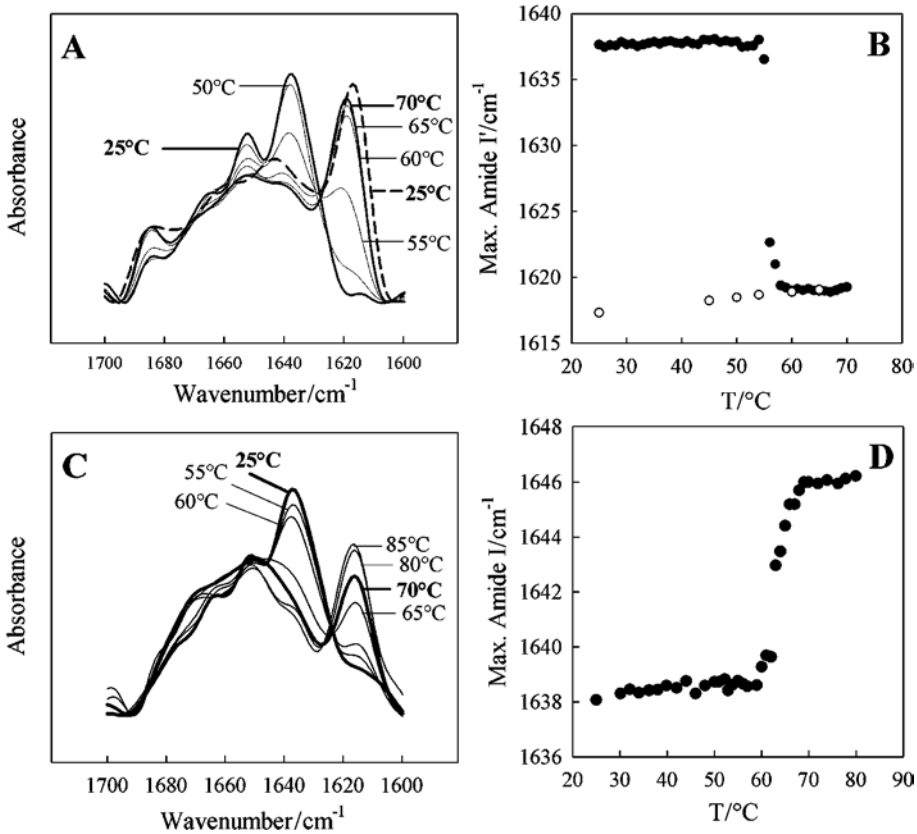

Figure 4 Temperature stability of PME at 0.1 MPa and 200 MPa

(**A**) Deconvoluted amide I' band of PME at atmospheric pressure and different temperatures (the full line at 25 °C is before heating, the dashed line after cooling); (**B**) maximum of the amide I' band as a function of the temperature during heating (●) and cooling (○) at atmospheric pressure; (**C**) Deconvoluted amide I' band of PME at 200 MPa and different temperatures; (**D**) maximum of the amide I' as a function of the temperature during heating (●) at 200 MPa.

FTIR during thermal treatment (Figure 4). The amide I' band of the native protein, at 25 °C, exhibits a prominent band at approx. 1638 cm^{-1} (β-helix) with a small shoulder at approx. 1652 cm^{-1} (α-helix) (Figure 4A). Increasing the temperature to nearly 50 °C induces no significant changes in the spectrum. The onset of unfolding and consequent aggregation starts at above 50 °C. The intensity of the band at approx.1638 cm^{-1} begins to decrease, becomes broader and shifts to a slightly higher wave number (1645 cm^{-1}) indicating that the protein begins to unfold. At elevated temperatures this becomes more prominent, and apart from this a band at approx. 1619 cm^{-1}, accompanied by a less intense band at approx. 1682 cm^{-1}, appears. These bands point to the formation of intermolecular anti-parallel β-sheet aggregation [25]. Temperature unfolding resulted in amorphous aggregation due to the high concentrations needed to perform FTIR spectroscopy measurements. A plot of the maximum of the amide I' as a function of the temperature clearly shows the onset and the transition of the process occurring in a narrow temperature range between 55 °C–60 °C (Figure 4B). Owing to the aggregation, temperature unfolding is irreversible as can be seen from the open dots in Figure 4(B). The IR spectra clearly demonstrate that the loss in enzyme activity during heating was due to a change in the overall conformation of the enzyme. This correlation between enzyme inactivation and protein unfolding as observed by FTIR or other techniques has previously been observed in many other enzymes [26–28].

It is important to keep in mind that protein unfolding and enzyme inactivation are kinetic phenomena, not only governed by temperature but also by time. In other words, decreasing the treatment time will increase the magnitude of the remaining enzyme activity. Equally, the onset-temperature of the protein unfolding can be shifted to slightly higher values when the heating rate is increased.

Although there is a large difference between the concentration used in the inactivation experiments and that necessary in FTIR spectroscopy, there is a good correlation between the inactivation and the unfolding temperature. Variation in the concentration used in FTIR spectroscopy was hard to achieve, since a lower concentration would give rise to an IR signal that was too low. However, higher concentrations were not feasible due to the low solubility of the enzyme. The small difference in temperature can be assigned to the difference in protein stability in D_2O compared with H_2O. It has been shown that in the case of ribonuclease A, lysosyme and cytochrome *c* dissolved in D_2O, the transition temperature is shifted to a slightly higher value compared with experiments performed in non-deuterated water [29]. Based on differential scanning calorimetry and pressure perturbation calorimetry studies Sasisanker et al. [30] suggested that this difference in stability is caused not only by stronger and more compact hydration in D_2O, but also by a more compact protein conformation in deuterated solvents. Therefore performing the experiments in D_2O instead of H_2O can cause a small difference between the transition temperatures obtained in inactivation experiments performed in non-deuterated solvent, and in FTIR measurements performed in deuterated solvent.

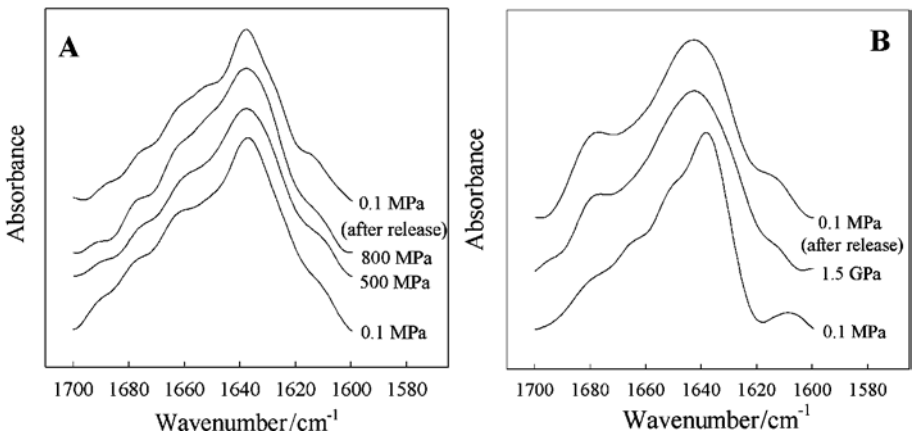

Figure 5 Deconvoluted amide I' band for PME at 25 °C and different pressures

(**A**) Pressure is increased up to 800 MPa and then released. (**B**) Pressure-increase up to 1.5 GPa.

Effect of pressure on PME stability at room temperature

Pressure treatments up to 700 MPa at room temperature did not significantly inactivate recombinant *Aspergillus aculeatus* PME. After a 15 min treatment at 20 °C and 200 MPa, 400 MPa or 700 MPa, PME still exhibits 100 % of its activity (Figure 3), confirming previous findings [9]. In the DAC pressures above 700 MPa, the pressure limit of the equipment used for the inactivation studies, could be reached. IR spectra for PME were recorded during pressure treatments up to 1.5 GPa. In Figure 5(A) the amide I' band of PME is shown at atmospheric pressures of 500 Mpa, 800 MPa and at atmospheric pressure after pressure treatment. The spectra confirm the pressure stability of the enzyme since no significant changes in the spectra could be observed following pressure increases of up to 800 MPa (Figure 5A) and even up to 1 GPa (results not shown). This indicates that PME does not unfold within the pressure range (400–800 MPa) wherein the majority of proteins undergo pressure-induced unfolding [31]. However, when pressure is increased above 1 GPa unfolding takes place, as can be seen in Figure 5(B) by the broadening of the band and the shift from approx. 1638 cm^{-1} towards approx. 1645 cm^{-1}. This one-step process is irreversible, since the band did not shift back to its original position and remained rather broad after pressure release. It is reasonable to assume that the change in the overall conformation happening in that pressure range will affect the enzyme's active site and cause irreversible inactivation. However, it cannot be ruled out that within the lower pressure range some minor changes (not affecting the secondary structure components, as the amide I' region remains unaffected up to 1 Gpa) take place, as the inactivation experiments are only performed after pressure treatment. Nevertheless, if such changes occur, they are reversible, since no effect on the activity due to pressurization can be detected.

Effect of combined pressure–temperature treatments on PME stability

An antagonistic effect of pressure and temperature on recombinant *Aspergillus aculeatus* PME stability was reported previously [9]. At 55 °C and pressures of 100 MPa and higher, hardly any enzyme inactivation was observed [9]. The results presented in Figure 3 confirm the protective effect of pressure against thermal inactivation of PME, since no inactivation is detected after 15 min treatment at 200, 400 and 700 MPa at 55 °C. Also at increased temperatures there is an antagonistic effect of pressure and temperature on PME stability, although some PME inactivation can be detected. At 400 and 700 MPa, high pressure protects PME against inactivation during a 15 min treatment at 60 °C. However, after 15 min at 200 MPa and at 60 °C 10 % of PME activity is lost. A treatment at 65 °C causes activity loss at all pressures studied. At 0.1 MPa, 200 MPa, 400 MPa and 700 MPa respectively 0 %, 43 %, 79 % and 72 % of the initial activity is retained after 15 min at 65 °C. The increased protein stability towards thermal inactivation at high pressure is also reflected in the FTIR spectra of the temperature scan at 200 MPa (Figure 4C). It is shown that at higher pressures the onset of unfolding is shifted to a higher temperature. Comparing the spectra at 60 °C and atmospheric pressure (Figure 4A) with that at 200 MPa (Figure 4C), it is shown that at atmospheric pressure the band at approx. 1619 cm^{-1}, indicative of intermolecular aggregation, was already quite intense. In contrast at 200 MPa, this band has only just started to appear when the band at approx. 1638 cm^{-1}, indicative of the native structure, has only just begun to decrease. The same observation can be made when considering the maximum of the amide I' at these two different pressure values (Figure 4B and 4D). The first difference that can be noted is that the onset of the transition begins at a higher temperature when pressure is increased. Furthermore, at atmospheric pressure, the maximum is shifted immediately towards approx. 1619 cm^{-1}, the band indicative of aggregation, whereas at 200 MPa the maximum of the band has moved towards approx. 1645 cm^{-1}, characteristic of unordered structure. This can be explained by the fact that pressure (counteracting hydrophobic forces to a certain extent [32]) has a suppressive effect on the aggregation of the partially unfolded protein.

It is not the first time such an observation has been made. Lipoxygenase, α-chymotrypsin and β-galactosidase for example exhibit a similar behaviour; they all demonstrate the protective effect of pressure against thermal inactivation in the lower pressure region [26,33,34] but the pressure effect in the case of PME is unusually high (ca 6 °C/100 MPa). The fact that at low pressure the protein is stabilized against temperature unfolding is reflected in the elliptic phase diagram [35]. A similar shape was obtained in the pressure–temperature diagram for inactivation of carrot PME [36]. Meersman et al. [37] have recently discussed the possibility that pressure-induced stabilization towards temperature-induced unfolding is related to the effect of pressure on protein aggregation.

The unusual pressure stability of PME

Whereas the temperature of unfolding in PME is within the normal range for mesophilic proteins, the pressure stability is unusually high. Another enzyme with an unfolding pressure of 1.2 GPa is HRP (horseradish peroxidase) [38]. There are only very few proteins with comparable or even higher pressure stability. One of them is the BPTI (bovine pancreatic trypsin inhibitor) [39], which is stabilized by two disulphide bonds. Disulphide bonds, however, are not always needed for high stability, as has been shown for the P2 protein from the archaeon *Sulfolobus solfataricus* [40,41]. However it may be significant that both BPTI and P2 are small proteins. HRP, on the other hand, has 306 amino acid residues. The PME studied in this work, with its 321 residues, provides a second example of a larger protein showing such extreme pressure stability.

In the case of PME the unusually high pressure stability may be connected to the presence of a substantial amount of protein in β-helix conformation. This is supported by the similarly high resistance to pressure inactivation reported for tomato PME [42] and carrot PME [36], the two plant PMEs for which a right-handed parallel β-helix crystal structure was reported. Also pectate lyase, another protein characterized by a parallel β-helix conformation [43], has been shown to be highly resistant towards denaturant-induced (guanidinium chloride) unfolding [44] and temperature unfolding [45].

Several authors have pointed to the possibility of the parallel β-helix as the molecular make-up of other highly pressure-stable protein structures such as amyloid [8,46,47]. The model for amyloid is consistent with solid-state NMR data, X-ray diffraction patterns and hydrogen/deuterium exchange experiments using amyloid fibrils. Furthermore, when regarding sequence specificity, there is no sequence fingerprint for the right-handed parallel β-helix, achieved by native proteins like the pectinases [15], nor for peptides or proteins that are prone to amyloid formation [48]. However, for technical reasons, no definitive proof that β-helical structures exist within amyloid has been obtained to date. In the present study, we wish to point out the correlation between the high pressure stability of recombinant *Aspergillus aculeatus* PME and the high pressure stability of mature amyloid fibrils, arising from $TTR_{105-115}$-peptide [10]. Pressure increases up to 1.2 GPa were not able to induce structural changes in the fibrils. Although the model for the amyloid fibril is very similar to the parallel β-helix in the native fold there are still some differences. One major difference lies in the fact that in native proteins the β structure is interrupted by non-β structure at several places in the sequence. Whether this could be the cause of the somewhat lower (but still high) stability of PME compared with mature amyloid fibrils remains to be established.

CONCLUSION

FTIR spectroscopy was applied to analyse the secondary structure of native PME and to monitor the overall conformational changes during pressure–temperature treatments. The correlation between the conformational protein-stability towards pressure and temperature analysed by FTIR and the enzyme's functional stability, assayed by inactivation experiments, was excellent within experimental error. FTIR studies revealed that the native enzyme structure is dominated by β-helix and clearly starts to unfold at temperatures higher than 50 °C. This was reflected by the loss of catalytic activity due to thermal treatment at 50 °C or higher temperatures. It was confirmed that the enzyme is very stable towards pressure. No significant inactivation was detected after treatment at moderate temperatures and pressures up to 700 MPa. Pressures above 1 GPa had to be applied to observe a change in the overall enzyme conformation. Moreover, high pressure protected PME against thermal denaturation. With increasing pressure the temperature necessary to induce unfolding of the structure and inactivation of the enzyme shifted to higher values. The protective effect of high pressure on the stability of fungal PME creates possibilities for the combined application of the exogenous enzyme and high pressure processing to create food products with novel functionalities. The unusual pressure stability of PME is assigned to the presence of β-helices. The high pressure stability of these enzymes supports the β-helix model that has been proposed for amyloid fibrils.

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