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Effect of High Hydrostatic Pressure on the Secondary Structure of Microbial Transglutaminase

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Abstract: Enzyme activity and corresponding secondary structure, measured by circular dichroism was analysed before und after treatment of microbial transglutaminase at different temperatures (40, 80°C) and pressures (0.1, 200, 400, 600 MPa). Irreversible enzyme inactivation was achieved at 80°C after 2 minutes at atmospheric pressure. Enzyme inactivation at 0.1, 200, 400, 600 MPa and 40°C followed first order kinetics. Increasing pressure reduced MTG activity, nevertheless the enzyme showed a residual activity of 50% after 12 min at 600 MPa. The analysis of the native enzyme exhibited well-defined proportions between α-helix, β-strand, β-turn and unordered structures. In contrast to heating, high-pressure treatment only at high levels induced significant decrease in the α-helix content, whereas β-strand substructures remained unaltered in both cases. Based on the known crystal structure of MTG it can be concluded that the active centre of the enzyme itself, which is located in an expanded β-strand domain, is relatively stable and pressure-induced inactivation is caused by a degradation of α-helix elements with corresponding influence on the tertiary structure.

Keywords: high pressure; secondary structure; microbial transglutaminase

INTRODUCTION

In recent years, food and drink manufacturers have applied high-pressure processes in order to satisfy consumers demand for additive-free foods and products with natural, unchanged sensory attributes. In this technology the destruction of vegetative microorganisms in the food is achieved with minimal heating, thus leading to maximum conservation of vitamins, natural colours and flavours while preserving the quality of the food. Some effects of this technology are changes in the functional properties of proteins such as emulsifying capacity, gelling, foaming, activation or inactivation of enzymes and unfolding of their secondary, tertiary and quaternary structures. The dissociation of oligomeric proteins is favoured at moderate pressure (< 150 MPa) whereas pressures higher than 150–200 MPa induce unfolding of proteins and re-association of subunits from dissociated oligomers [1]. Since a microbial transglutaminase (MTG) is available from fermentation processes, the enzyme has been approved for use in the food industry by many countries as a processing aid to improve quality of different food products. The enzyme transglutaminase (protein-glutamine γ-glutamyltransferase, EC.2.3.2.13) catalyses acyl transfer reactions between γ-carboxyamide group of peptide-bound glutaminyl residue and a variety of primary amines [2]. Previous studies have shown that MTG is remarkably stable under high-pressure treatment, enabling the simultaneous application of MTG and high pressure processing. The objective of this work was to study the conformational changes of the secondary structure of MTG after high hydrostatic pressure treatment in relation to enzymatic activity.

EXPERIMENTAL

Materials. Transglutaminase Active MP isolated from *Streptovercillium mobaraense* was supplied by Ajinomoto Co. Inc. (Hamburg, Germany). Chemical used were of highest purity available.

Methods. A hydrostatic pressure plant (Bernd Dieckers GmbH, Willich, Germany) was employed in this study. High-pressures were generated using a hydrostatic pump and water-ethylene-glycol mixture. Samples of MTG (5 ml) in polypropylene tubes were placed in the preheated pressure vessels of the hydrostatic pressure machine and were treated at 0.1, 200, 400, 600 MPa for 10–60 min at 40°C and other samples at 60, 70 and 80°C for 2, 4, 6, 8 and 10 min. After each treatment, enzymatic activity of MTG was measured [3] and CD spectra of the enzyme were recorded. Far-UV circular dichroisms (CD) of the samples were recorded in the range of 178 to 260 nm in buffer at a protein concentration of 0.2 mg/ml using a Jasco J 710 spectropolarimeter (Gross-Umstadt, Germany). The CD spectra were analysed by a curve-fitting software CDPro using CONTIN method as described by Sreerama and Woody [4], to obtain the secondary structural contents of the proteins. The estimation was performed using a 43-protein reference set [4].

RESULTS AND DISCUSSION

Thermal and high-pressure inactivation

The thermal inactivation of MTG at atmospheric pressure in a temperature range between 60–80°C was measured. An inactivation of 89% was observed after heating at 60°C for 5 min and complete inactivation was achieved at 80°C for 2 min. Activity was not reversible at 37°C over a period of 180 min. High hydrostatic pressure inactivation at 40°C in a pressure range between 0.1–600 MPa was analysed (Figure 1).

MTG inactivation followed a first-order kinetic model. Rate constant values (k) at 0.1, 200, 400 and 600 MPa were 0.0047, 0.0097, 0.018 and 0.047 min^{-1}, respectively and showed that inactivation velocity increased with higher pressure. When the rate constants for enzyme inactivation at 40°C were plotted as a function of pressure, an inactivation volume of –9 cm^3/gmol was obtained. These values correspond closely with those reported by Lauber *et al.* [5]. The inactivation volume showed that MTG was more stable than phosphatase (–58 cm^3/gmol), glutamyltransferase (–65cm^3/gmol) or α-amylase (–45 cm^3/gmol) at 40°C [5].

Estimation of structure by circular dichroism

MTG solutions were treated at 40°C for 30 min at 0.1, 200, 400 and 600 MPa. To obtain complete inactivation, other samples were treated at 40°C for 60 min and 80°C for 2 min. After high-pressure or thermal treatment, the samples were centrifuged to separate the irreversibly denatured protein. Soluble protein was analysed by circular dichroism spectroscopy. Spectra in the far ultraviolet wavelength range or amide region (190 to 260 nm) were used to characterise the conformation structure of the enzyme (Figure 2). Native MTG showed a

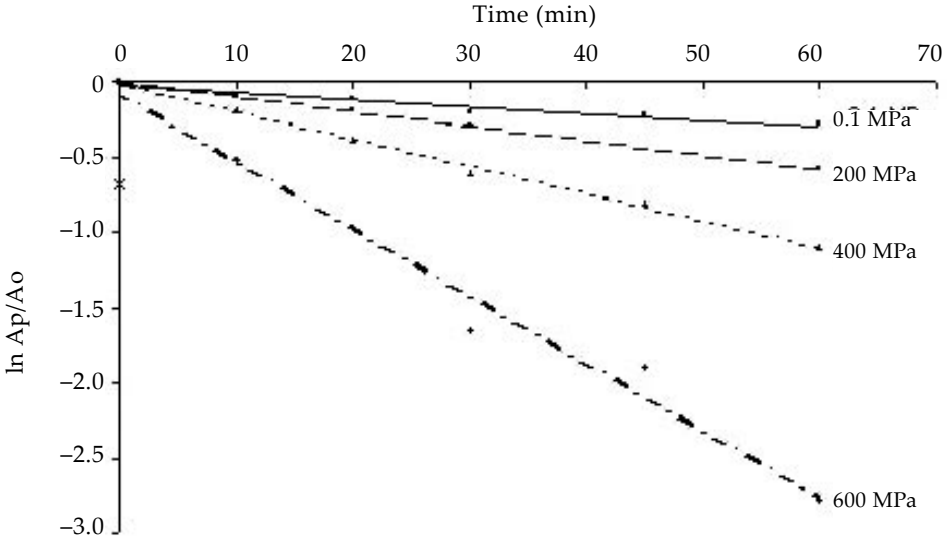

Figure 1. Inactivation of MTG by high-pressure treatment at 0.1, 200, 400 and 600 MPa and 40°C

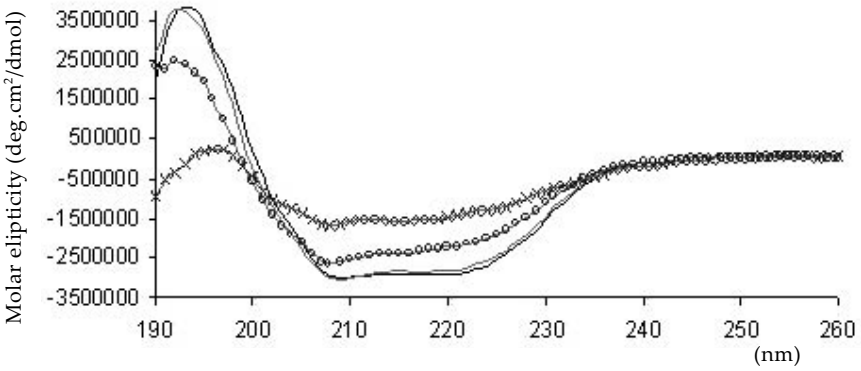

Figure 2. Far-UV CD spectra of MTG at different temperatures and pressures. Native (-), 400 MPa, 30 min at 40°C (--), 600 MPa, 60 min at 40°C (-o-), 80°C, 2 min (–+–)

Table 1. Secondary structure of MTG after high pressure and thermal treatment

Treatment	H*	S*	Turn*	Unrd*
1. MTG – test	24.50	23.80	21.05	30.65
2. MTG – 40°C – P atm – 30 min	23.50	23.60	21.00	31.90
3. MTG – 40°C – 200 MPa – 30 min	24.20	22.90	21.00	31.90
4. MTG – 40°C – 400 MPa – 30 min	23.30	23.90	21.20	31.50
5. MTG – 40°C – 600 MPa – 30 min	19.70	25.90	20.90	33.50
6. MTG – 40°C – 600 MPa – 60 min	17.20	27.50	20.55	34.75
7. MTG – 80°C – 2 min	6.50	28.20	18.40	46.90

*H = α-helix; S = β-strand; Turn = β-turn; Unrd = unordered

typical band of the α-helix. n-π* band at ~222 nm and the π-π* band split in to two transitions, namely a π-π* (⊥) band at ~191 and a π-π* (II) band at ~208 [6]. The decreased cotton effect at 222 nm reflects alterations in the secondary structure and could suggest that treatment above 600 MPa at 40°C during 30 min caused drastic changes to the α-helix MTG conformation. A short thermal treatment at 80°C for 2 min was sufficient to achieve the highest loss of the original structure.

The calculation of the secondary structure content of the enzyme was performed using a CONTIN method from far-UV CD spectra (Table 1). The native enzyme consisted of 54.50% α-helix, 23.80% β-strand, 21.05% β-turn and 30.65% unordered structure. The loss of α-helix and the increased unordered structure occurred after high pressure and thermal treatment. After treatment at 600 MPa and 40°C for 60 min, the content of α-helix decreased to 17.20%. Massive destruction of α-helix to a residual amount of 6.5% was achieved after thermal treatment at 80°C for 2 min. Tauscher [7] reported that β-sheet structures are nearly incompressible and more stable against pressure than α-helix structures. The tertiary structure of MTG is arranged so that active centre, located in a β-strand domain is surrounded by α-helices [8].

Pressure induced degradation of this conformation would primarily take place at these α-helical areas on the molecule's surface, leading to an alteration of the tertiary structure with subsequent consequences on substrate binding.

We therefore suggest that the enzyme activity under elevated pressures is closely related with the relative stability of α-helix and the outstanding stability of the central β-strand structure.

CONCLUSIONS

MTG showed high stability towards high-pressure treatment. Whereas thermal treatment at 80°C for 2 min resulted in a significant destruction of α-helical regions of the proteins concomitant with a complete inactivation, the α-helix content decreased only slightly after treatment at 600 MPa and 40°C for 60 min. As the content of β-strand regions was

not influenced by both physical treatments, the remarkable stability of MTG under high-pressure can be explained with the stability of the active site which is located within an extended β-strand region of the protein. This property of MTG may make it a valuable aid for *in situ* food processing under high pressure.

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