Deamidation of Several Food Proteins Using Free and Immobilized Ca$^{2+}$-Independent Microbial Transglutaminase

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Enzymatic deamidation of $\varepsilon_{1}$-casein was done by using Ca$^{2+}$-independent microbial transglutaminase (MTGase) of a variant of Streptoverticillum mobaraense. Although the amount of deamidated glutamine residues in $\varepsilon_{1}$-casein was not as high as that of the case using guinea pig liver transglutaminase (GTGase), the improvements in pH-solubility and Ca$^{2+}$-sensitivity profile of the substrate protein were comparable to it. To do the enzymatic deamidation without chemical acylation of Lys residues of $\varepsilon_{1}$-casein, several immobilized MTGase were prepared with two types of chitosan beads. Although neither $\varepsilon_{1}$-casein nor $\beta$-casein was deamidated, dimethyl casein and citraconylated soy 7S globulin were deamidated by using the immobilized enzymes.

Key words: deamidation; transglutaminase

Chemical and enzymatic deamidation of food protein has been studied by several researchers.\(^1\)\(^-\)\(^4\) Transglutaminase is an enzyme that can cause glutamine-specific deamidation.\(^5\) Motoki et al. showed that guinea pig liver transglutaminase (GTGase) deamidated $\varepsilon_{1}$-casein in combination with a reversible acylation of the $\varepsilon$-amino groups of lysine residues to suppress self-polymerization.\(^6\) Ca$^{2+}$-independent microbial transglutaminase was found as an extracellular product of a variant of Streptoverticillum mobaraense.\(^7\) The abilities of the enzyme in preparing protein gels have been also reported.\(^8\)\(^-\)\(^10\) The first objective of this paper is to show the capability of MTGase in deamidation of food proteins. On the other hand, the reversible acylation used in our previous work\(^6\) to suppress self-polymerization of $\varepsilon_{1}$-casein seems to be unfavorable for food manufacturing. Therefore, the second objective is to attempt the preparation of covalently immobilized MTGase and study the effects of immobilization on protein deamination.

MTGase (31 units mg) was prepared from the culture medium of a variant of Streptoverticillum mobaraense by the method previously described.\(^7\) Enzyme activity was measured by the hydroxamate procedure with CBZ-glutamyl glycine.\(^11\)

Twelve grams of chitosan beads (Chitopearl 3007, 3010, Fuji Bouseki, Tokyo, Japan) were thoroughly washed with distilled water, then suspended in 2.5% (w/v) gluteraldehyde (20 ml) and incubated for 120 min at room temperature (22°C). After four washings with distilled water, the beads were mixed with 15 ml of 0.1% (w/v) MTGase solution (50 mM phosphate buffer, pH 7.0) and incubated for 120 min at room temperature (22°C). Then, the beads were washed with 1 M NaCl two times followed by three washings with distilled water. To inactivate the excess aldehydes, the beads were mixed with 20 ml of 0.1 M ethanol amine solution (50 mM phosphate buffer, pH 7.5) and incubated for 60 min at room temperature (22°C). After a washing with distilled water, the immobilized MTGase was obtained and kept in 50 mM phosphate buffer (pH 7.0).

Citraconylated $\varepsilon_{1}$-casein and soy 7S globulin were prepared by the method described in our previous work.\(^8\) The degree of acylation of citraconylated $\varepsilon_{1}$-casein as well as citraconylated soy 7S globulin was 96%. $\beta$-casein and dimethyl casein were purchased from Sigma Co. (St. Louis, MO).

Citraconylated $\varepsilon_{1}$-casein was dissolved to make 1.0% (w/v) in 50 mM Tris-HCl buffer (pH 7.5). Then, MTGase (0.1 units mg substrate protein, heat-inactivated MTGase was used for the control) was added to the solution, and the mixture was incubated for four hours at 37°C. After the incubation was finished, the pH of the reaction mixture was adjusted to 3.5 with 1 N HCl and kept for three hours at room temperature (22°C) to decitraconylate the substrate protein as previously described.\(^8\)

The extent of the deamidation was measured from the number of ammonium ions caused by MTGase reaction. A sample (1 ml) of the reaction mixture was mixed with 10% (w/v) trichloro acetic acid and centrifuged to remove the protein fraction. Then, 2 M K$_2$CO$_3$ (0.25 ml) was mixed with a sample (1 ml) of the supernatant and 0.2 ml of the mixture was put into the F-kit ammonia (Boehringer Mannheim, Germany). For other substrates, 1.0% (w/v) solutions (50 mM Tris-HCl, pH 7.5) were prepared. Then, free or immobilized MTGase (0.04 units mg substrate) was added to the reaction mixture and incubated for two hours at 37°C.

Tissue if any polymerization of the substrate protein occurs, several samples were analyzed on SDS-polyacrylamide gel electrophoresis under reducing conditions, as described in our previous work.\(^6\)

The pH-solubility profile and Ca$^{2+}$-sensitivity of $\varepsilon_{1}$-casein were analyzed by the method described in our previous work.\(^6\)

It was confirmed by SDS-polyacrylamide gel electrophoresis that citraconylated $\varepsilon_{1}$-casein was not polymerized during the MTGase reaction while almost all intact $\varepsilon_{1}$-casein was polymerized. On the other hand, it was found that about 1.15 mol of ammonium ions were released from one mole of citraconylated $\varepsilon_{1}$-casein. This means that about 7.7% of the glutamine residues of $\varepsilon_{1}$-casein were deamidated. This value of deamidation is much lower than the value (79%) reported for GTGase applied to $\varepsilon_{1}$-casein.\(^8\) As we mentioned in our previous work, there seemed to be a difference between MTGase and GTGase in terms of substrate specificity.\(^7\) Therefore, it is not so unreasonable that MTGase deamidated citraconylated $\varepsilon_{1}$-casein less than GTGase. Despite the low rate of deamidation, the effects of deamidation caused by MTGase was clearly shown on pH-solubility and Ca$^{2+}$-sensitivity (Figs. 1 and 2). The deamidated $\varepsilon_{1}$-casein seems more soluble in the pH range 5.0 to 5.5 than the control (Fig. 1). This result is comparable to that observed in the deamidated $\varepsilon_{1}$-casein prepared by using GTGase.\(^8\) Meanwhile, the deamidated $\varepsilon_{1}$-casein shows lower Ca$^{2+}$-sensitivity than the control (Fig. 2). In the range of calcium ion concentration higher than 12 mM, the deamidated $\varepsilon_{1}$-casein was as well soluble as it was in the solution without calcium. It was also newly found that MTGase could deamidate dimethyl casein and citraconylated soy 7S globulin (shown in Table I).

Abbreviations: MTGase, Ca$^{2+}$-independent microbial transglutaminase; GTGase, guinea pig liver transglutaminase.
On two types of chitosan beads, BCW3007 ($\phi=0.7$ mm) and BCW3010 ($\phi=1.0$ mm), MTGase was covalently immobilized and had the enzyme activity (measured by the hydroxamate method\cite{11}) at 9.6 and 8.8 units g wet beads, respectively. By using free and immobilized MTGase, deamidation of intact $\alpha_1$-casein and $\beta$-casein were tried. Figure 3 shows that self-polymerizations of $\alpha_1$-casein and $\beta$-casein were inhibited in the sample incubated with the immobilized MTGase instead of free MTGase. But it was also found that there were no significant releases of ammonium ions for both substrates. To see the ability of immobilized MTGase in deamidation with other substrates, dimethyl casein and citraconylated soy $7S$ globulin were used. As is summarized in the Table, the immobilized enzyme deamidated the substrates although the amount of released ammonium ions were lower than that caused by the free enzyme. The result suggests that there may be steric hindrances around the active centers of the immobilized enzymes, so small peptides like CBZ-glutaminyl glycine (substrate for the enzyme activity measurement) can reach the active center of MTGase. Although the primary structure of MTGase has been described\cite{12}, the tertiary structure around the active center of MTGase is still unknown. It should be figured out for future study to obtain useful immobilized MTGase for protein deamidation.

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\textbf{References}