

Enhancement of Halophilicity and Thermophilicity of Thermolysin by Modification with Polyethylene Glycol and Preparation of Functional Peptides from Food Proteins

Kuniyo Inouye (Kyoto University, Graduate School of Agriculture)

Toshiyuki Sakaki (Kyoto University), Teisuke Takita (Kyoto University), Motoki Kubo (Ritsumeikan University), and Koichi Morimoto (Kinki University)

Thermolysin (TLN) is a thermophilic and neutral metalloproteinase produced from the culture medium of *Bacillus thermoproteolyticus*. We have reported that the activity of TLN is enhanced remarkably in the presence of high concentration of salts and that TLN is a typical halophilic enzyme. The TLN activity of hydrolysis and synthesis of furylacryloyl (FA)Gly-Leu-amide (FAGLA) and carbobenzyloxy (Z)Asp-Phe-OMe (ZAPM) is enhanced 12-13 times by the addition of 4 M NaCl, and the activation is considered to be due to the change in the electrostatic environments of TLN. In the present study, modification of amino groups of TLN with polyethylene glycol (PEG) is performed and the effects of PEG on the activity and thermo-stability are examined.

MATERIALS AND METHODS. Amino groups on the surface of TLN were modified by 2,4-bis(*O*-methoxypolyethylene glycol)6-chloro-*s*-triazine (mPEG₂; 10 kDa). Because no lysyl residues are located in the active site of TLN, the modification and introduction of PEG moieties to TLN might not show direct influence to the active site.

RESULTS AND DISCUSSION. The TLN activity in the FAGLA hydrolysis was enhanced depending on the number of mPEG₂ moiety introduced into TLN. The modified TLN (5.2PEG-TLN) in which 5.2 amino groups out of 12 were modified with PEG showed the activity 5.7 times higher than that of TLN. The activity of TLN and 5.2PEG-TLN increased with increasing [NaCl] in an exponential fashion, and the degree of activation of 5.2PEG-TLN was 5.6 at 4 M NaCl. The activity of both TLNs showed bell-shaped pH dependence with pK_a values of 5.2 and 8.0, suggesting that activation of TLN by PEG was not derived by pK_a shift of the ionizable residues of the active site. The activation of the FAGLA hydrolysis by PEG modification was followed by the changes in K_m and k_{cat} values. On the other hand, the activity of the ZAPM hydrolysis was inhibited noncompetitively by the PEG modification. The degree of the inhibition was enhanced by NaCl. The thermostability of TLN was also enhanced by the PEG modification, and T_{50} value (75.9°C) of TLN was changed to 79.6°C in 5.2PEG-TLN.