Effect of the maillard reaction on the bactericidal activity of ε-polylysine

Journal of Tokyo University of Fisheries, Vol. 84, No. 2, pp. 25–30, December 1997

EFFECT OF THE MAILLARD REACTION ON THE BACTERICIDAL ACTIVITY OF ε-POLYLYSINE*

Ho Yu Ting*[1], Shoichiro Ishizaki*[1] and Munehiko Tanaka*[1]

The effect of the Maillard reaction on the bactericidal activity of ε-polylysine (PL), the natural food preservative, was investigated. The mixed solutions of PL and glucose with different ratio at pH 8–11 were heated at 40–100℃ for up to 120 min to accelerate the Maillard reaction. The rate of the reaction was monitored by the wavelengths at 283, 326, and 420 nm. Bactericidal activity of PL was determined by the dilution method and expressed as minimum inhibitory concentration (MIC, μg/ml).

The Maillard reaction between PL and glucose was significantly progressed at the pH range above 10 and was negligible below pH 8. The rate of the reaction was faster at higher heating temperature and larger ratio of glucose to PL. The activation energy for the development of the Maillard reaction was constant regardless of the pH level. PL had the bactericidal activity towards both Gram-positive and -negative bacteria. The Maillard reaction of PL did not significantly influence its bactericidal activity.

Key words: The Maillard reaction, ε-Polylysine, Bactericidal activity

Introduction

A variety of preservatives have been used to prolong the shelf-life of processed foods. However, with consumers becoming more health conscious and from the standpoint of food safety, there has been a trend toward processed foods with natural preservatives. Among natural preservatives, the usage ε-polylysine has been recently increasing because of its unique properties including potent antimicrobial activity (Otsuka *et al.*, 1992).

Shima and Sakai (1977) isolated a homopolymer of L-lysine, ε-poly-L-lysine (PL) with the structure described below, from the culture medium of *Streptomyces albulus*. A degree of polymeriza-

$$-(NH-CH_2-CH_2-CH_2-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-CO)_n-$$

tion (n) is 25–30 (molecular weight *ca.* 4,000). The peptide groups are connected with four methylene groups and one methine. Therefore, PL molecule has both hydrophobic and hydrophilic groups, which give PL the surface active property. Since the amino group of PL has a positive charge in an aqueous solution, PL behaves like a cationic surface active agent. Furthermore, it is known that PL disrupts outer membrane of Gram-negative bacteria and also kills these microorganisms (Shima *et al.*, 1984; Delihas *et al.*, 1995).

PL is a very effective natural antimicrobial agent, but there is a possibility of the occurrence of the Maillard reaction in processed foods between PL and reducing sugars, because PL contains a large number of free amino groups in its molecule. As a consequence of the Maillard reaction, undesirable phenomena such as development of brown color and off-flavor, losses of nutritional value and microbial activity might take place during processing with PL. Therefore, the present study was

* Received November 20, 1996.

*[1] Laboratory of Food Processing, Department of Food Science and Technology, Tokyo University of Fisheries, 5–7, Konan 4-chome, Minato-ku, Tokyo 108, Japan (東京水産大学食品製造学講座).

initiated to elucidate the effect of the Maillard reaction on the bactericidal activity of PL.

Materials and Methods

Preparation of the model systems

In order to facilitate the collection of the Maillard reaction data, model systems were used. The model systems used in this study consisted of ε-polylysine (Chisso, Co.) and glucose (Kokusan Kagaku, Co.). 0.5% PL solutions with various ratio (w/w) of glucose were used in all experiments. The ratios of PL to glucose were 1 : 0.1, 1 : 0.25, 1 : 0.5, 1 : 0.75, and 1 : 1. Both PL and glucose were dissolved in boric acid/NaOH buffers (pH 8–10) or $NaHCO_3$/NaOH buffer (pH 11).

Heating conditions

These sample solutions were heated under conditions as described below:

pH level: pH 8, 9, 10, 11

Heating temperature: 40–100℃

Heating time: 15, 30, 45, 75, 120 min

For heating purposes, 3 m*l* of the sample was placed in a vial (1.0 cm i.d.×4.2 cm) and hermetically sealed with a screw cap to minimize heat transfer limitations in come-up time. At each sample time, the samples were quickly placed into an ice bath.

Analytical procedures

The degree of the Maillard reaction between PL and glucose was monitored by measuring the absorbances at 283 nm, 326 nm, and 420 nm. The development of brown pigment was determined as absorbance at 420 nm. The absorbances at 283 nm and 326 nm of 20-fold diluted sample solutions were used to trace the progress of the Maillard reaction, because they increased with the heating time. The absorbances of the solutions consisting only of glucose were subtracted from those of PL and glucose mixed solutions in order to eliminate the effect of caramelization due to the heating of glucose. Free glucose in samples was determined enzymatically by the glucose B test (Wako Pure Chemical Ind., Ltd.).

Minimum inhibitory concentration (MIC, μg/m*l*) of PL was determined by the broth dilution method as follows; bacteria (Table 1) were inoculated into nutrient broth (Difco) with 1×10^6 cells/m*l* and aerobically incubated. Growth was recorded turbidimetrically after incubation at 30℃ for 48 h. MIC was recorded as the lowest concentration which completely inhibited the growth of bacteria. Each test was conducted in triplicate.

Results and Discussion

The development of the Maillard reaction between PL and glucose

Figure 1 presents the typical representative pattern of the progress of the Maillard reaction between PL and glucose as a function of pH at 80℃ (PL: glucose=1 : 1). Absorbances at 283, 326, and 420 nm increased during the reaction in a similar way for each model system, indicating that any of wavelengths can be used to trace the development of the Maillard reaction. Furthermore, it is obvious from Fig. 1 that the Maillard reaction between PL and glucose takes place quite rapidly above pH 10. Since most of foods we consume usually have neutral or acidic pH values, there are scarce possibilities of the Maillard reaction occurred due to the addition of PL to those foods.

Effects of heating temperature on the Maillard reaction between PL and glucose with the mixing ratio of 1 : 1 at pH 11 are shown in Fig. 2. The rate of the Maillard reaction was constant at the initial stage of the reaction and approached a plateau at the later stage, regardless of heating temperature. The induction period prior to visual detection of an increase in brown color was not observed or at least it was not detected within the accuracy of the determinations used in this study.

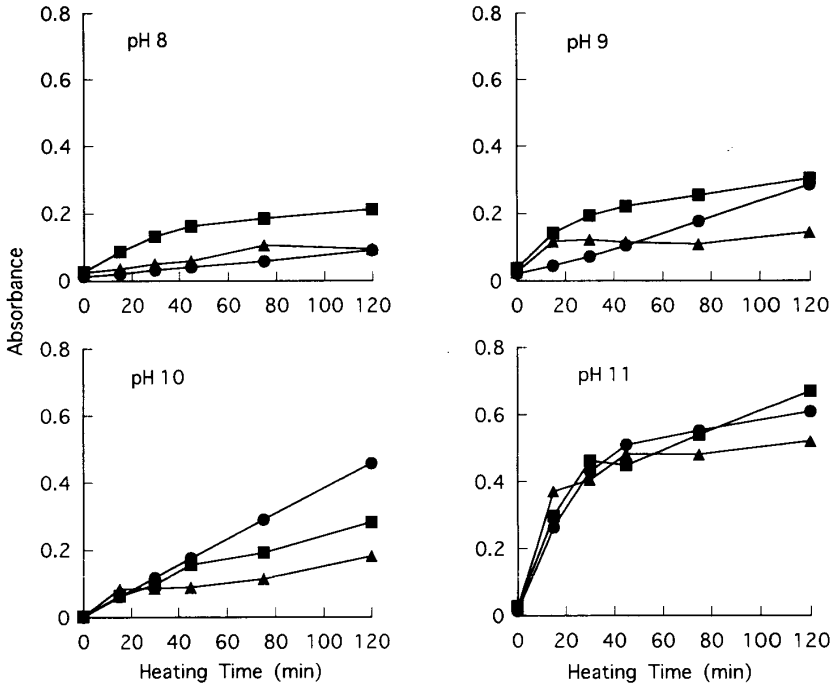

Fig. 1. Effect of pH on the development of the Maillard reaction (PL : glucose = 1 : 1) at 80℃. ■: 283 nm, ●: 326 nm, ▲: 420 nm.

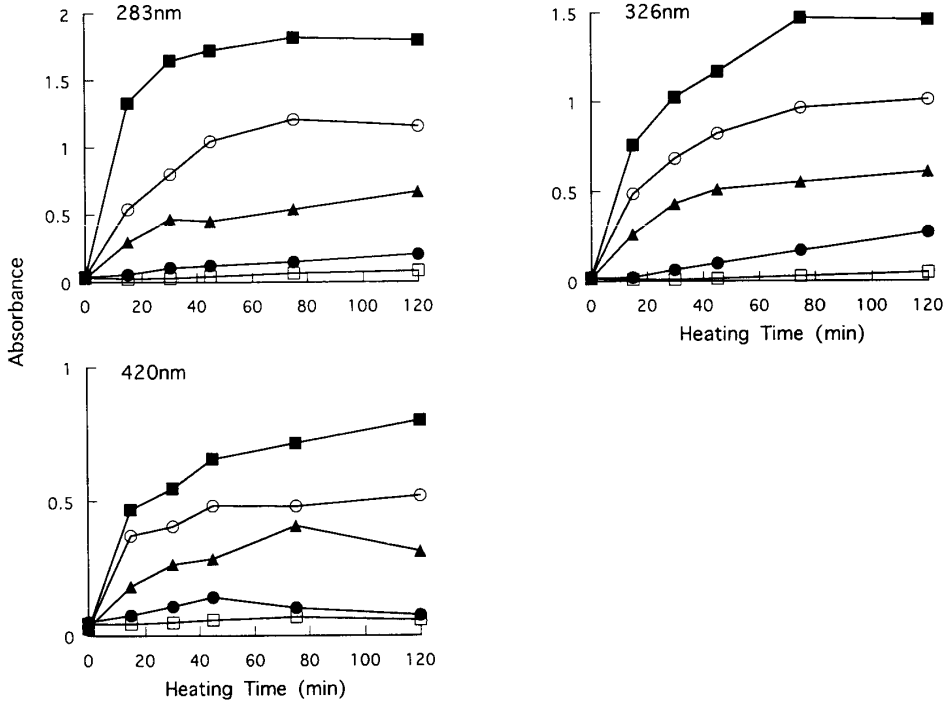

Fig. 2. Effect of heating temperature on the Maillard reaction between PL and glucose (1 : 1, pH 11). □: 60℃, ●: 70℃, ▲: 80℃, ○: 90℃, ■: 100℃.

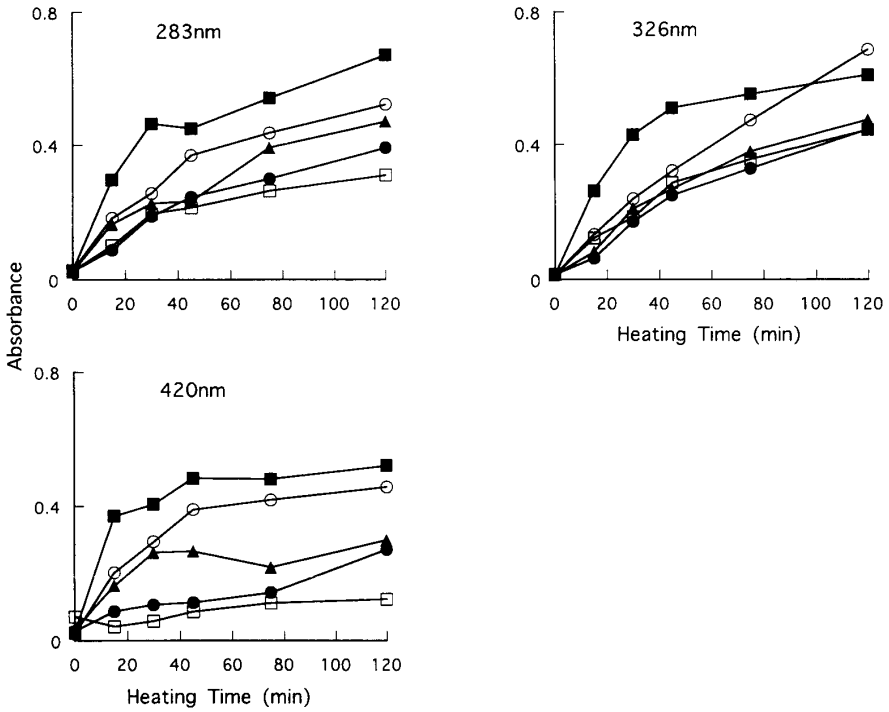

Fig. 3. Effect of ratio of PL to glucose on the development of the Maillard reaction at pH 11 and 80℃. □: 1 : 0.1, ●: 1 : 0.25, ▲: 1 : 0.5, ○: 1 : 0.75, ■: 1 : 1.

Figure 3 depicts the effect of ratio of glucose to PL on the development of the Maillard reaction at 80℃ and pH 11. It is apparent from this Figure that the rates of the Maillard reaction determined by the absorbances at 283 nm and 420 nm increased with the increasing ratio of glucose to PL. On the contrary, the amounts of free glucose significantly decreased at pHs 10 and 11 (Fig. 4), indicating that glucose was bound to PL by the Maillard reaction. These results are quite agreeable with those shown in Fig. 1.

The activation energies for the development of the Maillard reaction between PL and glucose were calculated from the Arrhenius plots of the rate constants ($\log k$) against $1/T$. The rate constants were obtained from the slopes of the linear portion of the Maillard reaction. Although detailed data are not shown, it was found that activation energies for the increases in absorbances at 283 nm and 420 nm were in the range of 24–25 kcal/mol, regardless of the pHs of the model system. On the other hand, the activation energy for the increase of absorbance at 326 nm was approximately 40 kcal/mol, regardless of the pH values. These imply that the reaction traced by absorbances at 283 nm and 420 nm is different from that by 326 nm.

Effect of the Maillard reaction on bactericidal activity of PL

Bactericidal activities of PL on different bacterial species have been revealed by Shima *et al.* (1984) and Delihas *et al.* (1995). According to the study of Vaara (1992), polycationic compounds disrupt the outer membrane of Gram-negative bacteria and PL disrupts the outer membrane by binding to it and discharging a large quantity of lipopolysaccharide. Since PL binds to the membrane through free amino groups of the molecule, there is a possible inhibition of the antimicrobial activity of PL as the result of the occurrence of the Maillard reaction.

Table 1 shows the bactericidal spectrum of PL and browned PL. Browned PL used in this study was prepared by heating the mixture of PL and glucose (1 : 0.75, pH 11) at 80℃ for up to 120 min. Bactericidal activity was measured by the dilution method.

PL had a wide bactericidal spectrum, inhibiting both Gram-positive and Gram-negative bacterial

growth at concentrations of 1–7.5 μg/ml except *Pseudomonas fluorescens* IAM 12022. MIC value of PL was in good agreement with the value reported by Shima *et al.* (1984). It is interesting to note that the MIC value varied with different strains of the same bacterium (*Staphylococcus aureus* IAM 12544 and IAM 1098), while MIC values against *Listeria monocytogenes* were identical between strains serotype 1/2a and serotype 4b.

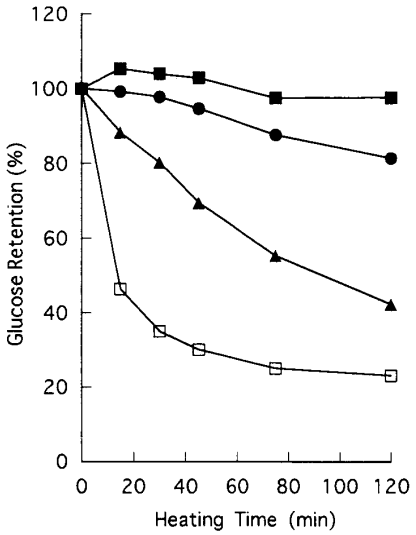

Fig. 4. Retention of glucose during the Maillard reaction between PL and glucose (1 : 1) at 80℃. ■: pH 8, ●: pH 9, ▲: pH 10, □: pH 11.

It is obvious from Table 1 that the initial Maillard reaction of PL with glucose proceeded at 80℃ and pH 11 did not influence its bactericidal activity against most of bacteria used in this study. However, prolonged heating resulted in a slight increase in MIC towards some of bacteria such as *Bacillus cereus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Pseudomonas putida*.

In conclusion, it was revealed that the Maillard reaction of PL did not take place at the neutral or acidic pH levels and the bactericidal activity of PL was not significantly influenced by the Maillard reaction with glucose even at high pH level. Further study for the effective utilization of PL in processed marine products is being

Table 1. Bactericidal spectrum of ε-polylysine and browned ε-polylysine.

Bacteria species		Minimum inhibitory concentration (μg/ml)					
		ε-Polylysine	Browned ε-polylysine*				
			15 min	30 min	45 min	75 min	120 min
Bacillus subtilis	IAM 1026	7.5	15	15	15	15	15
Bacillus cereus	IAM 12605	5	5	6	6	7	9
Listeria monocytogenes	serotype 1/2a	0.7	0.7	0.9	0.8	0.9	1
Listeria monocytogenes	serotype 4b	0.7	0.7	0.8	0.8	0.8	0.9
Staphylococcus aureus	IAM 12544	3	3	4	4	5	5
Staphylococcus aureus	IAM 1098	10	10	10	10	10	10
Escherichia coli	JCM 1649	3	3	3	3	3	3
Morganella morganii	Kono strain	2	2	2	2	3	3
Pseudomonas aeruginosa	IAM 1514	5	5	8	6	8	8
Pseudomonas fluorescens	IAM 12022	20	20	20	20	20	20
Pseudomonas putida	IAM 1236	5	6	6	6	7	8
Salmonella typhimurium	SH-1	3	3	3	3	4	4

* Browned ε-polylysine was prepared by heating the mixture of ε-polylysine and glucose (1 : 0.75, pH 11) at 80℃.

in progress.

Acknowledgment

The authors are grateful to the staff members of the Laboratory of Food Microbiology, Tokyo University of Fisheries for providing us the bacterial strains in addition to the technical support needed for this study. The authors also thank Chisso Co., Ltd. for providing us PL samples. This study was partly supported by the San-Ei Gen Foundation for Food Chemical Research.

References

Delihas, N., Riley, L. W., Loo, W., Berkowitz, J. and Poltoratskaia, N. 1995. High sensitivity of *Mycobacterium* species to the bactericidal activity by polylysine. FEMS Microbiol. Lett., **132**: 233–237.

Otsuka, N., Kuwahara, Y. and Manabe, M. 1992. Effect of ε-poly-lysine on preservation of boiled noodles. Nippon Shokuhin Kogyo Gakkaishi, **39**: 344–347.

Shima, S. and Sakai, H. 1977. Polylysine produced by *Streptomyces*. Agric. Biol. Chem., **41**: 1807–1809.

Shima, S., Matsuoka, H., Iwamoto, T. and Sakai, H. 1984. Antimicrobial action of ε-poly-L-lysine. J. Antibiotics, **37**: 1449–1455.

Vaara, M. 1992. Agents that increase the permeability of the outer membrane. Microbiol. Rev., **56**: 395–411.

ポリリジンの抗菌性に及ぼすメイラード反応の影響

何　玉婷・石崎松一郎・田中宗彦

天然保存剤として利用されているε-ポリリジン (PL) の抗菌性に及ぼすメイラード反応の影響について検討した。各種混合比 (w/w) の PL とグルコース溶液の pH を 8～11 に調整し，40～100℃で 120 分まで加熱した。メイラード反応の進行は 283, 326, 420 nm の吸光度を指標として追跡し，PL の抗菌性は Nutrient broth を用いる希釈法で求めた。

pH 10 以上でメイラード反応は急激に進行したが，pH 8 以下ではほとんど起らなかった。加熱温度が高く，グルコースが占める割合が大きいほど反応は進行した。メイラード反応の進行に対する活性化エネルギーは，pH に関係なく一定であった。PL および褐変化 PL はグラム陽性菌，陰性菌の発育を阻害し，メイラード反応は PL の抗菌性にほとんど影響を及ぼさないことが明らかになった。

キーワード：ポリリジン，メイラード反応，抗菌性