Inhibition by xylose–lysine Maillard Reaction Products of the Formation of MelIQx in a Heated Creatinine, Glycine, and Glucose Model System

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Mutagenic heterocyclic amines such as 2-amino-3-methylimidazo[4,5-f]quinoxoline (IQ) and 2-amino-3,4-methylimidazo[4,5-f]quinoline (MelIQ) were originally isolated from broiled and dried sardines,1,2 and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) was found in fried beef.3 MelIQx was formed in a model system by heating creatinine, glycine, and glucose.4 Besides, the mutagens IQ and MelIQ were also formed from a heated model system of creatinine, glycine, and fructose.5 These IQ compounds are among the most potent mutagens known, and are formed from a pyridine or pyrazine derivative, an aldehyde (or a related Schiff base), and creatinine.6 Kato et al.7 reported that the melanoidin formed from glucose-lysine was antimutagenic against mutagens such as 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P1), 2-amino-6-methylidipyrido[1,2-a:3,2-d]imidazole (Glu-P1), and IQ. Yen et al.8 have reported that most Maillard reaction products (MRPs), especially those of sugars with tryptophan and xylose with amino acids, strongly inhibited the mutagenicity of IQ, Trp-P1, and Glu-P1 in the presence of an S-9 mixture. In addition, MRPs derived from xylose and lysine had a good inhibitory effect on IQ toward Salmonella typhimurium TA98 and TA100.9 Apart from the antimutagenicity of sugar-tryptophan MRPs, tryptophan has been reported to decrease mutagenic activity in both models of heated glycine, creatinine, and glucose mixtures, and fried or boiled meat.10 Therefore, this study is to deal with the inhibitory effects of xylose-lysine MRPs on the formation of MelIQx in the model system made by heating creatinine, glycine, and glucose.

The xylose-lysine MRPs were prepared by refluxing D-xylose and L-lysine monohydrochloride (molar ratio, 1:2) at 100°C and pH 9.0 for 10 h.9 In a model system, glycine (7 mmol), creatinine (7 mmol), d-glucose (3.5 mmol), and different doses (1, 2, 5, and 10 mg) of xylose-lysine MRPs were refluxed in diethyl-glycol (210 ml) containing 14% (v/v) water at 128°C for 2 h.10 In a control group, no xylose-lysine MRPs were added.

The boiled mixture was diluted several times with distilled water, and chromatographed on an XAD-2 column (2.5 × 17 cm) to remove diethyl-glycol by washing with 600 ml of distilled water. The mutagenic fraction was eluted with 250 ml of methanol. The methanol fraction was evaporated to dryness in a vacuum. Then, this dried material was dissolved in 25 ml of 0.2 N HCl and extracted with dichloromethane. The aqueous fraction collected was adjusted to pH 12.0 with 8 N NaOH and re-extracted with dichloromethane. The basic dichloromethane layer was collected and then dried. It was dissolved with methanol and analyzed by Hitachi high performance liquid chromatography (HPLC). Conditions for HPLC were as follows: column, LiChrosorb RP-18 (5 μm, 125 × 4 mm); mobile phase, 0.01 M potassium phosphate buffer (pH 7.9); methanol (55:45, v/v); flow rate, 0.5 ml/min at ambient temperature. The elution was monitored with a UV-detector (254 nm). Identification of the peak of MelIQx was made by comparing the retention time to the results given and the conditions established by Jägerstad et al.11 There was only one peak observed between 7 and 13 min of retention time, and the retention time (9.5 min) of this peak coincided with that of the MelIQx peak as provided by Jägerstad et al.12 The MelIQx content was measured and expressed by the integrated peak area obtained in HPLC. Mutagenic activity of the basic extract was examined by the Ames test13 using S. typhimurium strain TA98 in the presence of S9 mixture. The Table shows the amount of MelIQx formed and the comparative mutagenicity of the basic extract in the systems with or without the addition of xylose–lysine MRPs. The MelIQx content formed in the control group is expressed as 100%. As 1 or 2 mg of xylose-lysine MRPs was added, the MelIQx content was reduced to 21.4% and 14.4%, respectively. When 5 or 10 mg of xylose-lysine MRPs was added, the formation of MelIQx in the model mixture was completely inhibited. When the dose of xylose-lysine MRPs is within 5 mg, the more xylose-lysine MRPs are added, the higher extent of the inhibition will be. On the other hand, the mutagenic activity of the basic extract without MRPs is also expressed as 100%. When 1 or 2 mg of xylose-lysine MRPs was added, the mutagenic activity of the basic extract was reduced to 93.2% and 87.2%, respectively.

In analyzing the results, when the MelIQx content is reduced, the decrease in mutagenicity of the basic extract does not correspond to the obvious decrease in the amount of MelIQx formed. This slightly decrease in mutagenicity may be caused by the presence of some mutagenic substances other than MelIQx in the basic extract. Conclusively, there is a trend of decreasing in mutagenic activity and MelIQx content as the dose of xylose-lysine MRPs increases. Therefore, there is an apparent correlation between the inhibition of MelIQx formation and the addition of xylose-lysine MRPs. In summarizing the above results, it is considered that the addition of xylose-lysine MRPs (1 mg) can inhibit the formation of MelIQx in the heated model mixture of creatinine, glycine, and glucose.

With respect to the report of Jägerstad et al.,12 some intermediates formed in the Maillard reaction have been proved to act as the precursors of mutagenic IQ compounds and other mutagen formation. It is considered that sufficient xylose-lysine MRPs might inhibit or interfere with the formation of certain Maillard reaction intermediates. Thus, further study is required to discover the mechanism of the mutagenic IQ compound formation via blocking or interfering with the Maillard reaction pathway.

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References
Inhibitory Effects of Maillard Products


