Short Communication

Isolation of 2-Amino-3-methylimidazo- [4,5-*f*]quinoline as Mutagen from the Heated Product of a Mixture of Creatine and Proline

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Mutagenicities of broiled fish and beef steak have been reported[1,2] and 2-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 2-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2),[3] 2-amino-dipyrido[1,2-*a*:3′,2′-*d*]-imidazole (Glu-P-2),[4] 2-amino-9*H*- pyrido-[2,3-*b*]indole (AαC), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (Me-AαC),[5] 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (Me-IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (Me-IQ_x)[6,7] have been isolated and identified in the cooked fish and meat as the mutagens. Some of the mutagens identified in the cooked fish and meat have an imidazole ring in the chemical structure, therefore creatine in the fish and meat may serve as the precursor to the formation of mutagenic imidazocompounds. We previously demonstrated that the heated products of the mixtures of creatine and glucose, one of creatine and fatty acids, and one of creatine and amino acids, exhibit mutagenic activity in *Salmonella typhimurium* TA 98.[8～11]

From these results, it is suggested that mutagenic imidazocompounds are formed by the reaction of creatine with the other compounds in fish and meat during cooking. In the present paper, in order to test the suggested hypothesis for the formation of mutagenic imidazocompounds, IQ was isolated and identified in the products of model systems consisting of creatine and the other compounds found in fish and meat such as glucose, fatty acids or amino acids.

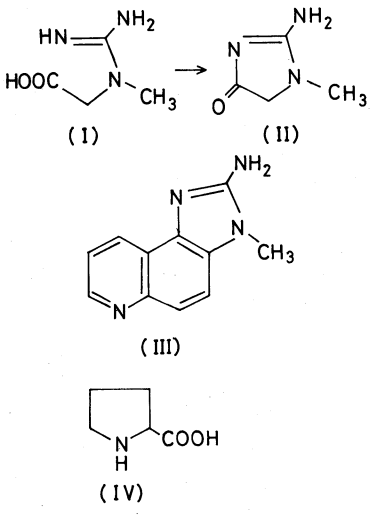
A mixture of 1.4 g of creatine and 1.8 g of glucose was heated in an electric furnace at 180°C under air atmosphere for 1 hr. The heated product was dissolved in water and extracted with ethyl acetate after adjusting to pH 11 by the addition of aqueous sodium hydroxide. IQ in the extract was separated by thin layer chromatography on Merck silica gel plates (20 × 20 cm) with a mixture of ethyl acetate and methanol (100 : 50, v/v) as the developing solvent. No spot corresponding to IQ was detected under UV light in the heated product of creatine and glucose.

A mixture of 1.4 g of creatine and 2.8 g of oleic acid was heated as described above. An aqueous solution of the heated product was adjusted to pH 2 and washed with ethyl acetate, and then extracted with ethyl acetate after adjusting to pH 11 by the addition of aqueous sodium hydroxide. IQ in the extract was separated. No spot corresponding to IQ was detected on the thin layer chromatography plate which was made as described above.

In the model systems for the heated products of the mixture of creatine and glucose or the one of creatine and oleic acid, other mutagens could have been produced.

Mixtures of 1.4 g of creatine and 10 mmol of each of cystine, threonine, phenylalanine, methionine, tryptophan, valine, proline, and serine were heated as described above and IQ was separated from the heated products. The amino acids indicated above exhibited higher mutagenic activity when they were heated with creatine, as reported previously.[8] The spot corresponding to IQ was detected on the thin layer chromatography plate on which the extract of the heated product of the mixture of creatine and proline had been applied.

To isolate IQ for the measurement of mass and UV absorption spectra, a mixture of 14 g of creatine and 11.5 g of proline was heated at 180°C for 1 hr and the heated product was



Structures of Creatine (I), Creatinine (II), IQ (III) and Proline (IV).

dissolved in water and adjusted to pH 11 by the addition of aqueous sodium hydroxide. The ethyl acetate extract of the aqueous solution was separated by thin layer chromatography on a Merck silica gel plate with a mixture of ethyl acetate and methanol (100 : 50, v/v) as the developing solvent. The spot corresponding to IQ was scraped off under UV light, and the methanol extract was again separated on a silica gel plate with a mixture of chloroform and methanol (100 : 40, v/v) as the developing solvent. The spot corresponding to IQ was scraped off and the methanol extract was subjected to high pressure liquid chromatography on a Bondapak C-18 column (3.9 × 300 mm) using a mixture of methanol and water (50 : 50, v/v) as the developing solvent at 1.5 ml/min of flow rate. A peak having the retention time of authentic IQ was obtained from the extract. The eluate corresponding to IQ was finally subjected to chromatography on a Sephadex LH-20 column (10 × 400 mm) using methanol as a solvent. The eluate corresponding to IQ was collected and the yield of IQ was approximately 5 μg judging from the peak height of IQ on high pressure liquid chromatography.

The mass spectrum obtained from isolated material showed fragment ion peaks at m/z 198 (100), 197 (24), 183 (23), 170 (9) and 156 (14) (relative intensities in parenthesis). These fragment ion peaks correspond satisfactorily to those of authentic IQ. The UV spectrum of the isolated material was similar to that of authentic IQ (λ_{max} nm in methanol: 213, 264, 354). The mutagenic activity of the isolated material was tested according to the procedure reported previously,[12] and found to be substantially the same as that of IQ.

It is known that proline as well as creatine are common components in fish and meat.[13,14] Therefore, it would be probable that IQ is formed from the reaction of creatine with proline during cooking. The formation of other mutagenic imidazo-compounds in the model experiment would be possible. The mechanism on the formation of IQ by heating the mixture of creatine and proline remains obscure.

Formation of IQ in the model system consisting of creatine, glycine and glucose proposed by Jägerstad *et al.*[15] was also tested. No spot corresponding to IQ was detected in the heated product of the model system.

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