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FORMATION OF MUTAGENS BY HEATING CREATINE AND GLUCOSE

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SUMMARY : The mutagenic activity toward Salmonella typhimurium TA 98 and TA 100 was investigated by heat treatment at temperatures up to 200°C of meat with identified components such as protein, adenine, creatine and a mixture of each of the 17 amino acids or glucose. Mutagenicity of these nitrogenous compounds was detected at the temperature of 150°C by adding glucose, consequently the yield of mutagenic activity by heating creatine and glucose was remarkably high. It is assumed that mutagens would be formed by the reaction of creatine and sugars during cooking of meat.

INTRODUCTION : Nagao et al(1) detected mutagens in the char from charcoal-broiled beef and fish, and Commoner et al(2), Spingarn et al(3) and Rappaport et al(4) have described the mechanism of forming of mutagens during the cooking of meat. Mutagenic principals were isolated from the heated beef extract and broiled fish by Spingarn et al(5) and Kasai et al(6),respectively. It has been considered that the mutagens are formed by the pyrolysis of protein. We already reported that the mutagens were formed by heating the protein at the temperature ranging from 200 to 700°C(7). Although surface temperature of meat in cooking may sometimes reach up to 200°C, it is known that the temperature of interior portion seldom exceeds 200°C. Spingarn et al(3) and Rappaport et al(4) reported that mutagenic activity was detected in the cooked meat at the temperature range up to 200°C. However, there exists no report at the present time on the pyrolytic formation of mutagens from protein at the temperature lower than 200°C. A series of experiments has been conducted to examine the possibility in the occurance of mutagenicity under such lower temperature.

MATERIALS AND METHODS

Test materials prepared for this study are albumin, adenine, a mixture of each of the 17 amino acids (amino acid mixture), creatine or glucose. One g of nitrogenous compound above indicated was heated in a glass flask

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which was kept in an electric drying oven with or without addition of 1 g of glucose. Heated sample was extracted with diluted hydrochloric acid and the basic fraction was extracted with chloroform after adjusting pH 11 with aqueous sodium hydroxide. The chloroform extract was evaporated to dryness in vacuo and the residue was taken up in dimethylsulfoxide to make 1 % concentration. Aliquots of these extracts were tested for mutagenicity. Beef and chicken meat were also heated after drying at 80°C and extracted with chloroform in the same manner above described.

Another group of chloroform extracts of the heated product of creatine and glucose or meats was subjected to chromatography on a Sephadex LH-20 column (2 x 40 cm) using methanol as solvent. Mutagenic fractions were further separated for thin layer chromatograph with Woelm silica gel plates (20 x 20 cm) by use a mixture of ethyl acetate and methanol (7 : 3, v/v) as the developing solvent. The spots having mutagenic activity were scraped off and the methanol extracts were again separated for thin layer chromatograph by use a mixture of chloroform and methanol (7 : 3, v/v). The spots having mutagenic activity were scraped off and the methanol extracts were subjected to chromatography on a Sephadex LH-20 column. Mutagenic fractions were evaporated to dryness in vacuo.

The mutagenic activity was assayed according to the Ames's method(8,9). A mixture of the tester strain, Salmonella typhimurium TA 98 (TA 98) and TA 100 (TA 100), test material, and S-9 Mix were poured onto an agar over-layer. After incubation at 37°C for 48 hrs, histidine revertant colonies were counted. Liver microsomal fraction (S-9) was prepared from rats treated previously by injecting polychlorinated biphenyl as described by Ames et al(9).

RESULTS AND DISCUSSION

Albumin, adenine, amino acid mixture, or creatine were heated with or without addition of glucose at 150°C, and the mutagenic activity of the heated products was examined. As shown in Table 1, after heating for 2 hrs, no mutagenicity was detected in the products of albumin, adenine or glucose, although lower activity yielded in the one of the amino acid mixture. However, it is significant that mutagenicity was detected only when glucose was added to these nitrogenous compounds in heat treatment. Further, the yield of mutagenic activity observed among these components was highest by heating of creatine and glucose.

All of test samples were assayed on both TA 98 and TA 100 with or without S-9 activation. The most responsive strain was TA 98 and the mutagens formed in this experiment required the metabolic activation with S-9 Mix.

Effect of heating temperature on the formation of mutagens in the heating product of creatine and glucose was examined. As shown in Table 2, no mutagenicity was detected at temperature up to 125°C, however detectable degree of mutagenicity was formed at temperature ranging from 150 to 250°C with peak occurance at 150°C.

Mutagenic principals in the heated product of creatine and glucose were separated by liquid-liquid partioning, and then prepared for column chromatograph with Sephadex LH-20 and for thin layer chromatograph with

Table 1.Mutagenicity of heated products of albumin, adenine, amino acid mixture and creatine with or without addition of glucose at 150°C

Heated substances	Heating time (h)	*Mutagenicity (His^+ revertants/g of nitrogenous compound)			
		TA 98		TA 100	
		+ S-9 Mix	- S-9 Mix	+ S-9 Mix	- S-9 Mix
Albumin + Glucose	1	0	0	0	0
	2	85	0	0	0
Adenine + Glucose	1	0	0	0	0
	2	462	0	0	0
Amino acid mixture + Glucose	1	3017	0	0	0
	2	2988	0	0	0
Creatine + Glucose	1	144320	0	8360	0
	2	249300	0	19530	0
Albumin	2	0	0	0	0
Adenine	2	0	0	0	0
Amino acid mixture	2	1332	0	0	0
Creatine	2	0	0	0	0
Glucose	2	0	0	0	0

* Each value is an average of 2 experiments. Spontaneous revertant colonies (TA 98,+S-9 : 39, TA 98, - S-9 : 29, TA 100, + S-9 : 171, TA 100, - S-9 : 184) have been subtructed.

silica gel plate. They are chromatographically distinguishable from benzo(a) pyrene, amino-α-carboline and amino-γ-carboline which are mutagenic principals in the pyrolysis products of protein(10). One of the mutagenic

Table 2. Effect of heating temperature on the mutagenicity of heated products of creatine and glucose

Temperature (°C)	Mutagenicity (His^+ revertants/g of creatine)
125	0
150	249300
175	207500
200	191500
225	62500
250	35000

Mutagenicity was assayed with TA 98 in the presence of S-9 Mix. The number of spontaneous revertants (39) was subtructed.

principals isolated yielded several thousands of revertant colonies per 1 μg. The value exceeds by 10 time of benzo(a)pyrene. Beef and chicken meat were heated at 150°C for 2 hrs and the mutagenic fractions were isolated according to the method described above. One of the mutagenic fractions produced by heating beef and chicken meats was chromatographically indistinguishable from those produced when creatine and glucose were heated.

Presence of creatine and sugars is common in meat while cook up at temperatures about 150°C, therefore it is assumed that the mutagenic substances as products of creatine and glucose would be contained in cooked meat. Studies leading to identification method of mutagens are underway.

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