Short Communication

Formation of Mutagens by Heating Creatine or Amino Acids with Addition or Fatty Acids

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We have reported that the mutagens are formed when creatine, arginine or lysine are refluxed with the addition of glucose for 8 hr¹⁾ and when the mixture of creatine and glucose is heated at the temperature ranging from 150 to 250°C.²⁾ It is assumed that the creatine plays an important role on the formation of mutagens during cooking of meat. Amino compounds readily react with fatty acids as well as sugars,^{3,4)} therefore it seems probable that creatine or amino acids would react with fatty acids in the meat during cooking and some of the reaction products may be mutagenic. In the present paper, we determined the mutagenicity of the reaction products of creatine or some nitrogenous compounds with the fatty acids at the temperature range on the cooking.

One g of creatine, a mixture of each of the 17 amino acids (amino acid mixture), adenine or albumin was heated for 1 hr with or without addition of 1 g of fatty acids or related materials, such as stearic, palmitic, linoleic and oleic acid or lard, beef tallow and soybean oil, in a glass flask which was kept in an electric furnace.

Heated samples were extracted with diluted hydrochloric acid and chloroform, and the aqueous phase was washed with chloroform. The basic fraction was extracted from the aqueous extract 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 dimethyl sulfoxide. Aliquots of these extracts were tested for mutagenicity.

The mutagenic activity was assayed according to the Ames's method.^{5,6)} 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 overlay. After incubation at 37°C for 48 hr, histidine revertant colonies were counted. Liver microsomal fraction (S-9) was prepared from rats treated previously by injecting PCB as described by Ames *et al.*⁶⁾

Effect of temperature on the formation of mutagens in the heating product of creatine and oleic acid was examined. As shown in Table I, detectable degree of mutagenicity was formed at temperature ranging from 100 to 250°C. The mutagenic activity was increased with increasing the heating temperature. Peak on the formation of mutagens by heating creatine with the addition of glucose occurred at such lower temperature as 150°C. 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 mutagen formed in this experiment required the metabolic activation with S-9 Mix.

Creatine was heated with the addition of various kinds of fatty acids at 200°C, and the mutagenic activity of the heated products was examined. As shown in Table II, after heating for 1 hr, no mutagenicity was detected in the products of creatine or fatty acids. Yamaguchi *et al.*^{7,8)} reported that autoxidized fatty acids show mutagenicity when sonicated with some detergents. However, it is significant that mutagenicity was detected when fatty acids, such as stearic, palmitic, linoleic and oleic acid, were added to creatine in heat treatment. The mutagenic activity of the reaction mixtures by unsaturated fatty acids such as linoleic and oleic acid was higher than those of saturated one such as stearic and palmitic acid. Mutagenic activity was also detected in the reaction mixture of creatine with lard, beef tallow or soybean oil, although the yields of mutagenic activity were lower than that of fatty acid above indicated.

Identified components such as protein, adenine, creatine or amino acid mixture in the

TABLE I. EFFECT OF HEATING TEMPERATURE ON THE MUTAGENICITY OF HEATED PRODUCTS OF CREATINE AND OLEIC ACID

Temperature (°C)	Mutagenicity* (His ⁺ revertants/g of creatine)			
	TA 98		TA 100	
	+S-9 Mix	−S-9 Mix	+S-9 Mix	−S-9 Mix
100	0.07×10^4	0	0	0
125	0.33 "	0	0	0
150	0.95 "	0	0	0
175	1.75 "	0	0	0
200	8.0 "	0	0.21×10^4	0
225	16.9 "	0	0.24 "	0
250	35.2 "	0	1.93 "	0

For details of sample preparation see text.

* The numbers of His⁺ revertants per plate were 1/10 of the value in the Table for the product at 100°C and 1/100 for the others with TA 98, and 1/50 for the products at 200 and 225°C and 1/100 for the one at 250°C with TA 100. Spontaneous revertants (TA 98, +S-9 Mix: 33, TA 98, −S-9 Mix: 28, TA 100, +S-9 Mix: 185, TA 100, −S-9 Mix: 197) have been subtracted.

TABLE II. MUTAGENICITY OF HEATED PRODUCTS OF CREATINE AND FATTY ACIDS AT 200°C FOR 1 HR

Heated substances	Mutagenicity* (His ⁺ revertants/g of creatine or fatty acids)
Creatine+Stearic acid	2.34×10^4
Creatine+Palmitic acid	3.64 "
Creatine+Linoleic acid	17.3 "
Creatine+Oleic acid	8.0 "
Creatine+Lard	0.50×10^4
Creatine+Beef tallow	0.45 "
Creatine+Soybean oil	0.22 "
Stearic acid	0
Palmitic acid	0
Linoleic acid	0
Oleic acid	0
Lard	0
Beef tallow	0
Soybean oil	0
Creatine	0.11×10^4

* Mutagenicity was examined on TA 98, +S-9 Mix. The numbers of His⁺ revertants per plate were 1/100 of the value in the Table for the mixtures of creatine and fatty acids and 1/20 for the others. Spontaneous revertants indicated in Table I have been subtracted. No mutagenicity was detected on TA 98, −S-9 Mix, TA 100, + and −S-9 Mix, with the exceptions of reaction products of creatine and linoleic or oleic acid which revealed low mutagenicity on TA 100, +S-9 Mix.

TABLE III. MUTAGENICITY OF HEATED PRODUCTS OF NITROGENOUS COMPOUNDS AND OLEIC ACID AT 200°C FOR 1 HR

Heated substances	Mutagenicity* (His ⁺ revertants/g of nitrogenous compounds)
Creatine+Oleic acid	8.0×10^4
Amino acid mixture +Oleic acid	2.95 "
Adenine+Oleic acid	0
Albumin+Oleic acid	0.02 "
Creatine	0.11×10^4
Amino acid mixture	1.81 "
Adenine	0
Albumin	0.02 "
Oleic acid	0

* Mutagenicity was examined on TA 98, +S-9 Mix. The numbers of His⁺ revertants per plate were 1/100 of the value in the Table for the mixtures of oleic acid and creatine or amino acid mixture, and 1/20 for the others. Spontaneous revertants indicated in Table I have been subtracted. No mutagenicity was detected on TA 98, −S-9 Mix, TA 100, + and −S-9 Mix, with the exception of reaction product of creatine and oleic acid which revealed low mutagenicity on TA 100, +S-9 Mix.

meat were heated with or without addition of oleic acid at 200°C, and the mutagenic activity was examined. As shown in Table III, no or lower mutagenicity was detected in the prod-

ucts of adenine or albumin with or without addition of oleic acid. However, it is significant that the yields of mutagenic activity was increased when oleic acid was added to creatine or amino acid mixture in heat treatment.

Presence of creatine, amino acids and fatty acids is common in meat which is cooked up sometimes at temperatures about 200°C on the surface,^{9,10)} therefore it is assumed that the mutagenic substances as products of creatine or amino acids and fatty acids would be contained in cooked meat. We are now attempting to isolate and identify the mutagens in the reaction products.

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