Isolation of the Carcinogen IQ from Fried Egg Patties

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After semisolid patties were formed, the equivalent of 26 kg (26 kgE) of fresh eggs was fried at 325 °C. The surface crusts were trimmed off and extracted with acetonitrile/water. The organic bases were separated from this crude material and submitted to a fractionation procedure guided by the Ames mutagen assay. This purification consisted of successive column chromatography steps carried out on four types of stationary phases: LH-20, μ-Spherogel, PRP-1, and amino bonded-phase silica, in that order. Following the PRP-1 purification step, the mutagenic activity was resolved into two bands with roughly 30% of the activity in the earlier band. Additional purification on the amino column permitted analysis of this band by high-resolution mass spectroscopy, and the presence of IQ in the fraction was confirmed. The estimated minimum amount of IQ in the original field egg crusts produced under these high-temperature frying conditions is 1 μg, or 0.1 ppb based on the mass of the fried patties.

The mutagen 3-methyl-1,3,6-triazacyclopenta[α]-naphthalen-2-amine (also called IQ) recently has been shown to be carcinogenic in mice and rats (Ohgaki et al., 1984; Takayama et al., 1984). This compound was originally isolated from broiled sardines and has also been detected in the mutagenic products of fried ground beef and cooked beef extract (Takayama et al., 1984). We report here the first isolation of IQ from a nonmeat or nonfish food source: fried egg.

In the procedure for the isolation of IQ from fried egg patties we used two size-exclusion chromatography steps (one with an HPLC column), which improves our previously published methods (Bjeldanes et al., 1982a; Felton et al., 1984) for the difficult task of recovering trace amounts of mutagenic organic bases from cooked foods. We have shown in previous studies (Bjeldanes et al., 1982b) that extractable mutagens are formed when egg patties are fried at 225 °C and above. For the present work we used a higher frying temperature to increase the total mutagen yield.

EXPERIMENTAL PROCEDURES

After the whites and yolks of 436 fresh extra-large eggs (25.9 kg) were blended together, the egg patties to be fried were prepared by warming 60-mL portions of the mixture in Petri dishes (9-cm diameter, lightly sprayed with corn oil) at 88 °C for 2 h. The patties were fried for 4 min/side on a thermostated griddle set for 325 °C. The surface crusts from a total of 485 fried patties (11.2 kg) were trimmed off to give 5.1 kg of starting material for extraction. The extraction (see Figure 1) was performed by blending the crust with 10 L of acetonitrile/water (10:1 v/v) in a Polytron homogenizer (Brinkmann Instruments Co.). The mixture was filtered, and the insoluble residue was reextracted with the same solvent three more times using volumes of 8, 7, and 7 L.

The acetonitrile was removed by rotary evaporation, and the residual aqueous mixture was acidified to pH 2 with HCl and then extracted with 2 L of ethyl ether to remove organic acids and neutral compounds. The ether phase was discarded, and the extraction was repeated twice with 1.5-L portions of ether. The aqueous phase was then neutralized (pH 7) with NaOH and passed through a column of Amberlite XAD-2 (5 × 130 cm) that was previously packed in water and washed with acetonitrile, methanol, and acetone, two bed volumes each, before the final rinse with four bed volumes of water. The mixture was applied with a flow rate of 22 mL/min of water, and following addition of the sample volume (3.2 L), an additional 3 L of water was passed through the column and discarded. Then, the organic bases were recovered from the column with 8.4 L of acetone as the eluting solvent.

The crude mixture of organic bases (about 12 g) was stripped of acetone by rotary evaporation and applied to a column of Sephadex LH-20 (5 × 90 cm) packed in helium-sparged methanol/dichloromethane (3:2, v/v). The sample was chromatographed in three 4-g portions at a solvent flow rate of 2.5 mL/min. Using the Ames *Salmonella* mutagen assay as a guide, the active fractions from each run were pooled. After removal of solvent by rotary evaporation, about 3 g of active material was recovered.

The final stages of purification were facilitated by chromatography on HPLC columns attached to Model 110A pumps (Beckman Instruments). A size-exclusion HPLC column (μ-Spherogel, 0.77 × 30 cm; Beckman Instruments) with acetonitrile as the eluting solvent (1 mL/min) was used in the first fractionation. The sample was injected in 300-mg portions, and the active fractions collected were pooled to give 310 mg of material with more then a 6-fold increase in the specific mutagenic activity in this step.

The active fraction was submitted to reversed-phase chromatography on a Hamilton PRP-1 column (0.7 × 30 cm) using an acetonitrile/water gradient (10–30% acetonitrile in water at a flow rate of 3 mL/min; 1000 ppm of diethylamine added to each solvent). A distinct band of mutagenic activity was resolved under these conditions, accounting for about 30% of the total activity recovered. Repeating the chromatography on this band isocratically at 10% acetonitrile resolved no additional bands of mutagenicity but did provide additional separation from UV-absorbing nonmutagens.

The final purification of the resolved band (about 40 mg) was carried out on a Spherisorb 5 amino bonded-phase column (1 × 25 cm; Phenomenex, Rancho Palos Verdes, CA). The eluting solvent was 20% 2-propanol in hexanes (each modified with 1000 ppm of acetic acid) at a flow rate of 4 mL/min. Reinjection of the collected active fraction, this time using 15% 2-propanol in hexanes (modified with acetic acid as before), further purified the active peak. A portion of this band was submitted for high-resolution

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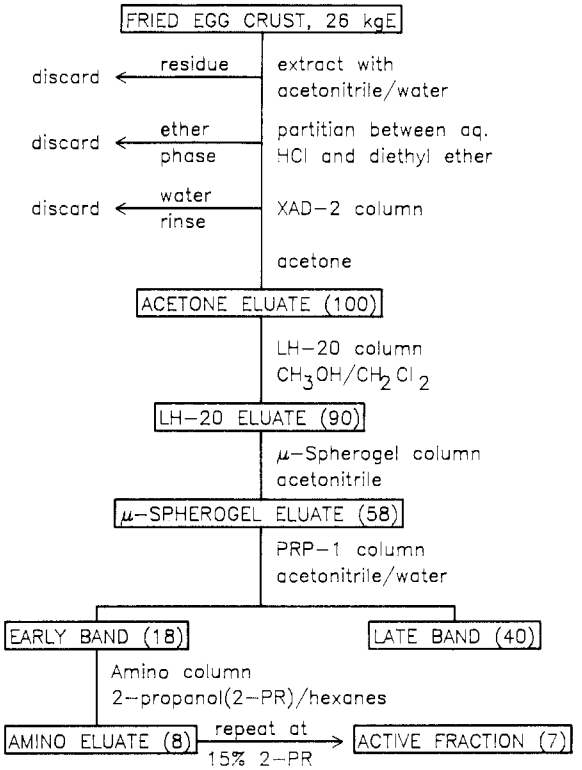

Figure 1. Extraction and purification procedure of a mutagen from fried egg patties showing results of the Ames mutagenesis assay (net revertants/gE values in parentheses) at each step. See text for details of the procedure.

mass spectral (MS) analysis (Varian MAT 311A).

RESULTS AND DISCUSSION

Following the chromatography of the active material on the amino column, it appeared that the amount of UV absorption and the total mutagenic activity in the fraction was consistent with about 1 μg of a mutagen having physical and biological properties similar to IQ. The active band coeluted with IQ standards injected under both reversed- and normal-phase analytical HPLC conditions. The MS analysis detected the six major ions also found with standard IQ samples (see Table I), confirming the presence of this compound in the egg extract. On the basis of the correspondence of peak area from the final chromatography and mutagenic activity of the isolated substance, the minimum amount of IQ in the original fried egg crusts is estimated to be 1 μg. This results in a concentration of 0.1 ppb based on the mass of the fried patties. The possible modulating effects on mutagenicity of other components in the egg crust prevent using the recovery of mutagenic activity (values in Figure 1) as a quantitative guide to correct for losses during the isolation procedure.

The conditions used in this study are not typical for the cooking of fried eggs. From our results one could assume, however, that the mutagenic activity we have observed (Bjeldanes et al., 1982b) under more reasonable cooking conditions is, at least in part, due to the compound IQ.

Table I. Mass Spectrum of the Mutagen from Egg

m/z^a	rel abund	ion formula[a]
199.0934	0.27 (0.37)[b]	$^{13}CC_{10}H_{10}N_4$
198.0907	2.90 (1.52)	$C_{11}H_{10}N_4$
197.0848	1.00 (1.00)	$C_{11}H_9N_4$
183	1.20 (0.90)	
170	0.60 (0.59)	
156	1.70 (0.78)	

[a] Fractional masses and ion formulas are based on high-resolution measurements. [b] Values in parentheses for authentic IQ.

The precursors of IQ-like compounds have been suggested (Yoshida and Fukuhara, 1982; Jagerstad et al., 1984; Taylor et al., 1985) as being creatine or creatinine in the presence of other amino acids and sugars. Our results comparing fried egg whites with egg yolks clearly implicate components found in the yolk as the precursors to the mutagens detected (Bjeldanes et al., 1982c). Assays of the water-soluble supernatant for one whole egg performed using the [α]naphthol–diacetyl method (Wong, 1971; Taylor et al., 1984) measured about 2 mmol/g of creatine/creatinine. This is about 15 times lower than the levels we have measured in ground beef but appears to be adequate to account for the formation of part-per-billion amounts of IQ-like compounds.

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