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ScienceDirect
Mutation Research 616 (2007) 90–94
Fundamental and Molecular Mechanisms of Mutagenesis
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Mutagenic potency of food-derived heterocyclic amines

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Available online 11 December 2006

Abstract

The understanding of mutagenic potency has been primarily approached using "quantitative structure–activity relationships" (QSAR). Often this method allows the prediction of mutagenic potency of the compound based on its structure. But it does not give the underlying reason why the mutagenic activities differ. We have taken a set of heterocyclic amine structures and used molecular dynamic calculations to dock these molecules into the active site of a computational model of the cytochrome P4501A2 enzyme. The calculated binding strength using Boltzman distribution constants was then compared to the QSAR value (HF/6-31G* optimized structures) and the Ames/*Salmonella* mutagenic potency. Further understanding will only come from knowing the complete set of mutagenic determinants. These include the nitrenium ion half-life, DNA adduct half-life, efficiency of repair of the adduct, and ultimately fixation of the mutation through cellular processes. For two isomers, PhIP and 3-Me-PhIP, we showed that for the 100-fold difference in the mutagenic potency a 5-fold difference can be accounted for by differences in the P450 oxidation. The other factor of 20 is not clearly understood but is downstream from the oxidation step. The application of QSAR (chemical characteristics) to biological principles related to mutagenesis is explored in this report.

Keywords: Heterocyclic amines; Mutagenic potency; QSAR

1. Introduction

In identifying the compounds responsible for the mutagenic potency of cooked meats, a series of aromatic amines were synthesized at Lawrence Livermore National Laboratory, USA [1], in Japan [2] and in Sweden [3]. Early on, it was surprising to note that small changes in molecule structure had a large impact on mutagenic potency in the Ames/*Salmonella* test. This was especially apparent in the frameshift sensitive *Salmonella* strains TA1538 and TA98 [4,5]. More than 20 mutagenic aromatic amines have been identified from food and additional closely related structural analogs and isomers have been synthesized to do structure–activity studies.

2. Mutagenic potency of cooked-food mutagens

There are two classes of aromatic amines related to cooked-food mutagens: amino-carbolines, and amino-imidazoazaarenes (AIA). A group of 23 amino-carbolines were evaluated for mutagenicity with a resulting range in mutagenic potency of over 200,000-fold. These studies found that potency depended on the

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doi:10.1016/j.mrfmmm.2006.11.010

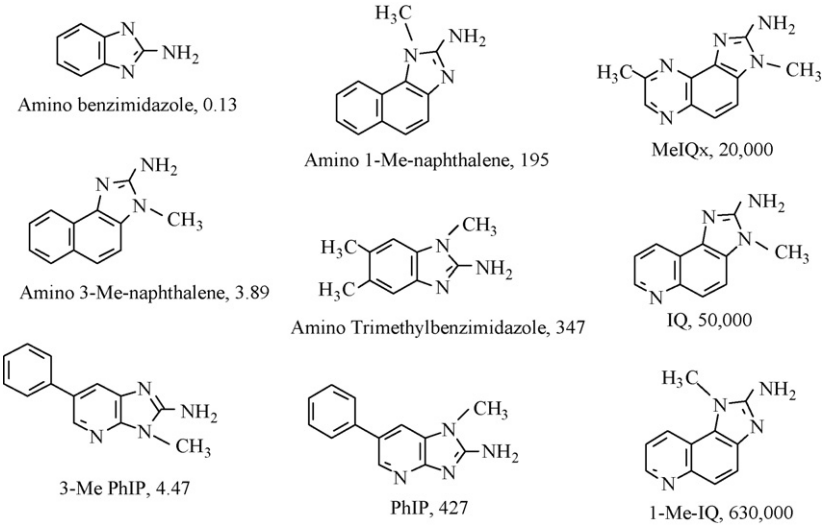

Fig. 1. Chemical structure and common name of aminoimidazoazaarene mutagens ranging 4.8 million-fold in mutagenic potency. Revertant colonies in *Salmonella* strain TA98 per nanomole are given.

presence and position of a pyridine-type nitrogen in one of the rings, the position of the amino group, and the methyl substitutions on ring carbon atoms [6]. The AIA class of mutagens comprises the most prevalent mutagenic compounds in well-done meat, and is therefore the most relevant for human exposures from foods. This class of mutagens is derived from creatine (a constituent of muscle), which contributes the amino group on the 2-carbon of the imidazole ring, common to these AIAs. These AIAs have mutagenic potencies ranging 4.8 million-fold as seen in Fig. 1. Three of the most commonly studied AIAs, PhIP, MelQx, and IQ, are found in well-done meat. The structural parameters that determine their mutagenic potency were determined to be: the number of fused rings, the number of heteroatoms in the non-imidazole ring, *N*-methyl substitution on the imidazole ring, and methyl substitution on ring carbon atoms [6]. Fig. 1 shows the more potent compounds have a heteroatom in the non-imidazole ring and have an *N*-methyl group. Although the chemical attributes of the mutagens can be defined, how this relates to the biology of mutagenesis is largely unanswered.

A set of 11 amino-trimethylimidazopyridine (TMIP) isomers, ranging 600-fold in mutagenic potency, were examined using computational methods. The principal determinants of higher mutagenic potency in these isomeric amines are: (1) a small dipole moment, (2) the combination of ring fusion and having the *N*3-methyl group, (3) a lower calculated energy of the pi electron system, (4) a smaller energy gap between the highest and lowest unoccupied molecular orbitals of the amine, and (5) and a more stable nitrenium ion [7].

A study of the relationship between the mutagenic potency in the Ames test and the carcinogenic potency in rodents of 34 aromatic amines shows that there is a significant quantitative relationship ($R = 0.66$, $P < 0.001$) between the two measurements after the results are translated into the appropriate quantitative terms [8]. Again, the chemical parameters of the number of rings in the compound and methyl substitutions at carbon atoms were important determinants in the carcinogenic potencies. These structure–activity studies were extended to the evaluation of electronic and hydropathic factors for 80 amines. The main determinant of mutagenicity was the extent of the pi electron system, with smaller contributions from dipole moment, the calculated stability of the nitrenium ion, and hydrophobicity [9].

3. Mutagenicity of two PhIP isomers

The importance of the position of the *N*-methyl group of PhIP is a good case study. The mutagenic potency of PhIP (methyl group at the 1-position) and its 3-Me-PhIP isomer differs by ∼100-fold in the Ames/*Salmonella* assay (Fig. 2A). None of the calculated chemical parameters mentioned above would explain the differences seen between these two isomers. We hypothesized that the slight differences in structure are important for interaction of the compound with the enzyme active sites for oxidation or further phase II enzymatic conjugation (see Fig. 2B). The data in Fig. 2C shows that the slower oxidation step (conversion of the PhIP isomers to their N–OH intermediate) from the 3-Me-PhIP isomer accounts for approximately 5-fold of the 100-fold dif-