Studies on the Oxidation Products from Phenylenediamine Derivatives as Hair Dye Ingredients by Hydrogen Peroxide and Their Mutagenicities

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The mutagenicity of hair dye components was first evaluated by Ames *et al.*¹⁾ using the *Salmonella*/microsome test. Mutagenicity of certain of these components was reportedly enhanced by H₂O₂-treatment, but the structures of the mutagenic oxidation products were not investigated.

In the present study, we report the mutagenicity of 13 kinds of *o*-, *m*- and *p*-PD derivatives, both before and after H₂O₂-treatment, in TA98 with or without S9 mix. The mutagenicity of *o*-PD, *p*-Cl-*o*-PD, *m*-PD, 2,4-diaminotoluene (*p*-Me-*m*-PD) and 2,4-diaminoanisole (*p*-OMe-*m*-PD) was remarkably enhanced by treatment with H₂O₂, and all oxidation products required metabolic activation by S9 mix for their mutagenesis. In a gas chromatography/mass spectrometric study, 2,3-diaminophenazine (2,3-diNH₂-Pz) and 2,7-diNH₂-Pz were identified with authentic samples in *o*-PD and *m*-PD oxidation mixture, respectively. The oxidation mixtures obtained from *p*-Cl-*o*-PD, *p*-Me-*m*-PD and *p*-OMe-*m*-PD were separated into several fractions by repeated column chromatography. 2,3-DiNH₂-7-Cl-Pz was isolated from oxidized *p*-Cl-*o*-PD, 2,7-diNH₂-3,8-diMe-Pz and 1,8-diNH₂-2,7-diMe-Pz were obtained from *p*-Me-*m*-PD, and 2,7-diNH₂-3,8-diOMe-Pz and 2,7-diNH₂-3-OMe-Pz were obtained from oxidized *p*-OMe-*m*-PD. The number of revertants induced by 1 nmol of phenazines detected from oxidized PD derivatives was as follows: 2,3-diNH₂-Pz 349 rev., 2,3-diNH₂-7-Cl-Pz 406 rev., 2,7-diNH₂-Pz 148 890 rev., 2,7-diNH₂-3,8-diMe-Pz 365 rev., 1,8-

diNH₂-2,7-diMe-Pz 49 rev., 2,7-diNH₂-3,8-diOMe-Pz 4229 rev., 2,7-diNH₂-3-OMe-Pz 24640 rev. in TA98 with 25 μl S9 per plate.

In order to determine the relationship between the structure of amino-substituted phenazines and their mutagenicity, we synthesized 8 species of aminophenazines and assayed their mutagenicity. Of 8 tested amino derivatives, 5 exhibited mutagenic activity with S9 mix in TA98. The order of mutagenicity of aminophenazines in TA98 was 1,7- < 2- < 2,8- < 2,3- < 2,7-substituted Pz. As regards the relationship between mutagenic potency and chemical structure of the phenazines, the results suggested that structural requirements favoring mutagenic activity were the presence of substituents at the 2 and/or 7 position.

In addition, for evaluation of the modulation effect of *p*-PD on the oxidation of *o*- or *m*-PD, the mixtures of *p*- and *o*-PD (*p*-PD/*o*-PD) and of *p*- and *m*-PD (*p*-PD/*m*-PD) were oxidized with H₂O₂ and their mutagenicity was tested in TA98 with and without S9 mix. The H₂O₂-oxidized *p*-PD/*o*-PD and *p*-PD/*m*-PD were potent mutagens with S9 mix and the major mutagenic oxidation products of *p*-PD/*o*-PD and *p*-PD/*m*-PD were identified by TLC and HPLC as 2,3- and 2,7-diNH₂-Pz, respectively. 2,8-DiNH₂-Pz was also found to be a reaction product in oxidized *p*-PD/*m*-PD, but it was slightly mutagenic in TA98 with S9 mix. The mutagenic potency of oxidized *p*-PD/*o*-PD was lower than that of oxidized *o*-PD. On the other hand, oxidized *p*-PD/*m*-PD was more mutagenic than oxidized *m*-PD, and it was considered that an unknown oxidation product(s) enhanced the mutagenic activity of 2,7-diNH₂-Pz in oxidized *p*-PD/*m*-PD, since no potent mutagens other than 2,7-diNH₂-Pz were detected.

Reference

- 1) B. N. Ames *et al.*, *Proc. Nat. Acad. Sci. USA*, **72**, 2423 (1975).