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One electron oxidation of 3-methylcholanthrene: A chemical model for its mechanism of carcinogenesis

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ABSTRACT

One electron transfer oxidation has long been proposed as a route to the ultimate electrophilic and carcinogenic metabolites of both methylated and non-methylated polycyclic aromatic hydrocarbons (PAH). The carcinogenic hydrocarbon 3-methylcholanthrene (3-MC) has a methyl-analogous function at its meso-anthracenic center in the form of a dimethylene bridge, and treatment of this compound with the one electron transfer oxidizing reagent ferric ferricyanide, $Fe^{III}(Fe^{III}(CN)_6)$, in mixed aqueous-organic media generated multiple oxygenated species, many of which duplicate those found in mammalian metabolism including known carcinogens 1-hydroxy-3MC and 1-keto-3MC. These results are in agreement with a Unified Theory for PAH Carcinogenicity which predicts *in vivo* generation of a proximate benzylic alcohol metabolite from the 3-MC procarcinogen and conjugation with a moiety such as sulfate intended for rapid urinary excretion. The sulfate instead acts as a leaving group and generates an electrophilic carbocation capable of reacting with sensitive nucleophiles such as DNA in cellular nuclei. The products of one electron transfer oxidation align well with predictions of the Unified Theory since in many cases these products provide substrates or precursors for conjugation reactions.

1. Introduction

Numerous studies have sought to identify the mechanism(s) by which specific polycyclic aromatic hydrocarbons (PAH) induce carcinogenesis [1–6]. With this end in view, it has been particularly important to determine the relative potency of oxidized metabolites arising from PAH metabolic activation pathways with diverse chemical endpoints. For example, some PAHs possess low ionization potentials and therefore readily form radical cations under oxidizing conditions. Wilk et al. [7] first proposed one electron oxidation of PAH as a mechanism to explain self-complexation of various PAH to dimers or tetramers visualized in UV and fluorescence spectroscopy studies. Fried & Schumm [8] and later Fried [9] picked up on the idea and treated carcinogenic PAH with $Fe^{III}(Fe^{III}(CN)_6)$ and related oxidizing agents and found oxidation products from the well-studied carcinogen 7,12-dimethylbenz[*a*]anthracene (DMBA) some of which—including 7-formyl-12-methylbenz[*a*]anthracene—were more active than the parent hydrocarbon in an *E. coli* phage inhibition system. These authors considered one electron oxidation as a potential mechanism for activation of some PAH to carcinogens. The carcinogen 3-methylcholanthrene (3-MC) formed a 1-hydroxy derivative that they considered active only as its 1-acetoxy derivative.

Studies of PAH metabolism by rat liver microsomes *in vitro* have suggested that oxygen-containing metabolites, such as those derived by one-electron transfer oxidation reactions and involving radical cation intermediates, may be particularly important for the metabolic activation and carcinogenicity of PAHs [3,10,11]. The potential biochemical significance of one electron oxidation for PAH carcinogenesis derives from the fact that reactive radical cation intermediates are involved in the processes that produce some of the same products formed by pathways occurring in animal cells. As a means of determining the relative importance of one-electron oxidation products in comparison to oxygen-containing metabolites of alternative pathways, we have sought to identify the oxidized products arising from a non-enzymatic model of one-electron oxidation of 3-MC for comparison with the known biologically oxidized metabolites of 3-MC by rat liver homogenates

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[12], the latter model having previously demonstrated itself capable of correlating carcinogenicity in PAHs with the occurrence of specific electrophilic substitution reactions.

The results show that the oxidized products arising from the one-electron oxidation model parallel and duplicate certain oxidized metabolites of 3-MC formed by rat liver homogenates. Since key oxidized products with pronounced carcinogenic activity are largely the same in the two systems, one may infer that the one-electron oxidation pathway is likely to occur to a significant extent in rat liver homogenates, and by inference in mammalian tissues, if one-electron oxidizing conditions are present. Certain reagents are capable of inducing one electron oxidation. The hexacyano-coordinated ferric ion appeared to be a suitable reagent as it is often treated as a model for heme-type oxidations such as carried out by heme-based cytochromes and other oxidases [13–15]. Since monooxygenation, for example via cytochrome P450s, is the major mechanistic pathway of oxidation in rat liver homogenates, one-electron oxidation is likely to play a role in producing specific oxidized metabolites with pronounced carcinogenic properties. The results, taken together with other observations, confirm predictions of the Meso-region Theory of PAH Carcinogenesis [4,16], also known as the Unified Theory, namely that products of monooxygenation such as benzylic alcohols and their exchange products such as halides or their conjugation products such as benzylic esters play a major role in carcinogenesis by 3-methylcholanthrene and related meso-methylbenz[*a*]anthracene derivatives, as depicted in Scheme 1. See Flesher & Lehner [17] for a review of this theory and its historical precedents.

2. Experimental section

2.1. Materials

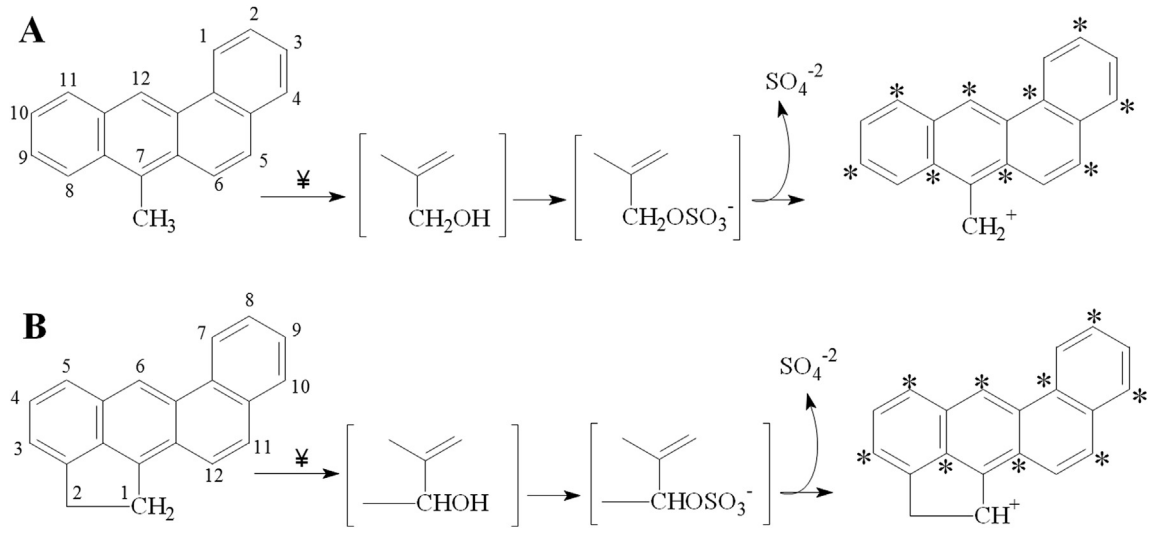
Hydrocarbon substrates were purchased from Sigma-Aldrich, St. Louis, MO (3-methylcholanthrene, 1-hydroxy-3MC, 3,6-dimethylcholanthrene). 1,2-Dehydro-3MC was prepared as described by Sims [12] starting with 3-MC. Compounds were recrystallized from appropriate solvents to achieve reported melting points and single peak purity by GC/MS wherever possible. Solvents (acetone, ethyl acetate, dimethylformamide) were HPLC-grade and were obtained from Fisher Chemicals (Waltham, MA).

2.2. Chemical one-electron oxidation

Reactions were carried out essentially as previously described [11] with some modifications. 3-MC, 1-hydroxy-3MC, 3,6-dimethylcholanthrene or 1,2-dehydro-3MC (0.2 mmol) was dissolved in 15 ml acetone. To this solution was added ferric ferricyanide $Fe^{III}(Fe^{III}(CN)_6)$ reagent generated from equimolar amounts of $FeCl_3$ (0.4 mmol; Baker Chemicals, Center Valley, PA) and $K_3Fe(CN)_6$ (0.4 mmol; Mallinckrodt, St. Louis, MO), dissolved in 5 ml water, a reagent known historically as Prussian blue [18]. The mixture was shaken in the dark in a water bath at 37 °C. for 16 h (mild conditions) or up to 96 h (stringent conditions). The reaction was stopped by addition of an equal volume of ethyl acetate, extracted by vigorous shaking for 10 min, and centrifuged to separate aqueous and organic layers. The extraction was repeated and organic layers combined. Combined extracts were washed with 5 ml water and centrifuged; this was repeated for a total of three washings. The washed organic solution was dried over anhydrous sodium sulfate. Aliquots (100–300 μl) of the organic phase were evaporated to dryness under a gentle stream of nitrogen and analyzed.

2.3. Gas chromatographic-mass spectrometric (GC/MS) analysis

Dried aliquots of the one-electron oxidation reaction or reference compounds were dissolved in 50 μl *N,O*-bistrimethylsilyltrifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS; Pierce Chemicals, Rockford, IL, now part of Thermo-Fisher, Waltham, MA) and derivatized by reaction at 75 °C for 30 min. Derivatized products and reference compounds were analyzed by GC/MS on an Agilent (Palo Alto, CA) 5890 GC equipped with a Zebron ZB-5MS column (30 m × 0.25 mm x 0.25 μm film thickness; Phenomenex, Torrance, CA) with a He flow rate of 1 ml/min. Standard operating conditions were as follows and referred to as the PAH.M method. The injector temperature was 250 °C; the GC oven was



Scheme 1. Principal routes of metabolism to carcinogenic species suggested by Meso-region Theory for 7-methylbenz[*a*]anthracenoid compounds (A) and for the structurally related cholanthrenoid compounds (B) such as 3-methylcholanthrene (3-methyl group not shown). Primary and secondary carbocations result from pathways A and B, respectively, and would go on to react with crucial and sensitive cellular nucleophiles such as nucleic acids along the pathway to carcinogenesis. Note that both routes A and B give benzylic mesoanthracenic carbocations. Either carbocation is stabilized by nine readily accessible resonance structures with the location of the resultant charges marked by the asterisks (*). These positions may benefit from further stabilization by methyl groups such as 3-methyl in 3-MC and 12-methyl in the strong carcinogen 7,12-dimethylbenz[*a*]anthracene. Reactions marked "¥" indicate ones expected to be facilitated *in vivo* by one electron oxidation.