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Transimination of Quinone Imines: A Mechanism for Embedding Exogenous Redox Activity into the Nucleosome

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Supporting Information

ABSTRACT: Aminophenols can redox cycle through the corresponding quinone imines to generate ROS. The electrophilic quinone imine intermediate can react with protein thiols as a mechanism of immobilization in vivo. Here, we describe the previously unknown transimination of a quinone imine by lysine as an alternative anchoring mechanism. The redox properties of the condensation product remain largely unchanged because the only structural change to the redox nucleus is the addition of an alkyl substituent to the imine nitrogen. Transimination enables targeting of histone proteins since histones are lysine-rich but nearly devoid of cysteines. Consequently, quinone imines can be embedded in the nucleosome and may be expected to produce ROS in maximal proximity to the genome.

Aminophenols principally to aminophenols through oxidative metabolism. This pathway is generally considered, at least with respect to carcinogenesis, as one of detoxification, but that assessment may represent a bias arising out of the understanding that N-, rather than ring-, hydroxylation of multicyclic aromatic amines is the key metabolic step in their activation to an ultimate carcinogenic form. Aminophenol formation is not necessarily the end point of oxidative metabolism if the substitution is ortho or para: it has recently been shown that these structures may be further transformed in vivo to quinone imines. Quinone imines are electrophiles and can undergo Michael addition with thiols and amines. They might, therefore, be capable of reacting with DNA nucleobases as a means of bringing about the mutagenic and carcinogenic effects observed with some alkylanilines such as 3,5-dimethylaniline, the focus of the present work. There is as yet no evidence for this mechanism of action.

In addition to their electrophilic character, 1,2- and 1,4-quinone imines are redox active structures that can cycle through the corresponding aminophenols to generate reactive oxygen species (ROS). As we have recently demonstrated with p-hydroxy-3,5-dimethylamine (3,5-DMA) and p-hydroxy-2,6-dimethylamine (2,6-DMA), aminophenols do generate ROS intracellularly. ROS generation in these experiments persisted for at least 24 h, indicating that the source was effectively immobilized within the cells. Immobilization could be achieved through Michael reaction with cellular thiols as such products have been shown to retain their redox activity, but this is not the only mechanism available to quinone imines. They are also capable of undergoing transimination, as described previously, whereby a primary amine displaces ammonia from the quinone imine. We demonstrate here that this occurs in cells with lysine residues. Histones, which are virtually devoid of cysteine residues, were of particular interest because lysine transimination of histones places the redox-active structure in the closest possible location to oxidizable DNA nucleobases and creates a potentially potent mechanism for genetic damage by aminophenols that is distinct from covalent modification of the bases.

Synthesis of the lysyl transimination product of 3,5-dimethylquinone imine (3,5-DMQI) is shown in Scheme 1. 3,5-DMAP was synthesized from 2,6-dimethylphenol as described for the isomeric 2,6-DMAP. To make [ring-14C]-3,5-DMAP, [ring-14C]-2,6-dimethylphenol was obtained by decomposition of [ring-14C]-2,6-dimethylbenzenediazonium salt.
ion in H₂O. Compound 1a was produced by reaction of 3,5-
DMQI, formed transiently by PbO₂ oxidation, with Nα-(t-
Boc)lysine. Following the transimination reaction, product 1a
was reduced with ascorbic acid to the more stable aminophenol
form Nα-(t-Boc)-Nα-(4-hydroxy-3,5-dimethylphenyl)lysine
(2a). With care to avoid oxidation, 2a could be isolated from
the reaction mixture by preparative HPLC, and its structure was
confirmed by 1D and 2D NMR and mass spectrometry. After
deprotection, 2b could not similarly be isolated because its
increased polarity did not permit separation from other
reactants. Nevertheless, it could readily be detected and
categorized by HPLC-MS (Figure 1A, upper trace, and
Figure 1B, upper spectrum). Reaction of unprotected lysine
with 3,5-DMQI apparently produced 1b since, after treatment
with ascorbate, the product was indistinguishable from 2b by
HPLC-MS.

Formation of Nα-(4-hydroxy-3,5-dimethylphenyl)-lysine (2b)
by histones in vivo was investigated using Chinese hamster
ovary AA8 cells grown in culture. Cells were treated with 25
μM 3,5-DMAP for 1 h, washed with fresh medium, and isolated
by centrifugation. Histones were isolated and digested with
protease in sodium acetate buffer containing 1 mM ascorbate
to inhibit oxidation. After initial purification by semipreparative
HPLC, 2b was analyzed by HPLC-MS using a triple-quad mass
spectrometer. Data acquired in multiple reaction monitoring
mode are shown in Figure 1A, which shows traces produced by
synthetic 2b as well as isolates from control histones (not
treated with 3,5-DMAP) and histones from treated cells. Figure
1A demonstrates cochromatography of histone isolate with
synthetic 2b; Figure 1B demonstrates that the CID spectra are
identical, confirming that the product isolated from histones is
indeed the lysine transimination product.

As evidence that 2b originated from histone protein and not
from any other protein that might have contaminated the
histone preparation, cells were treated with [14C]-3,5-DMAP
and the isolated histone fraction was subjected to HPLC for
analysis of isotopic labeling. The chromatographic separation
described by Boyne et al. for top down MS characterization of
histones was replicated for the present study, and the results
are given in Figure 2 with identification of the histone peaks as
given in the cited reference. In developing this separation, we
confirmed that the indicated peaks had masses appropriate for
histones but could not confirm the assignments made by Boyne
because of the complexity associated with post-translational
modifications. Quantitation of [14C] was performed on collected
fractions by accelerator mass spectrometry rather than decay
counting to achieve the requisite sensitivity. Figure 2 reveals the
correspondence of [14C] concentration with histone fractions
generally as well as two notable features that may be indicative
of site-specificity in the labeling reaction. The arrow marked
with an asterisk at 60.5 min indicates a region of the chromatogram where histone H2AZ elutes. With the exception
of one other fraction, this region of the chromatogram exhibits
the highest levels of [14C]. Histone H2AZ appears to be the
target of more extensive acetylation than other variants and,
by implication, might be more highly targeted by 3,5-DMAP,
thereby accounting for the higher degree of labeling relative to
the more abundant histones. The second notable feature of
Figure 2 is the [14C] peak at 63.2 min that corresponds to a
minor peak observed in the UV trace. The identity of this peak
is unknown, so we can only speculate that it represents a
hydroxydimethylphenylated histone variant. Alternatively, it
might correspond to phenylated H4 whose retention on the
HPLC column was increased by the modification.

The transimination reaction described here, between a
quinone imine and a primary amine, is not unknown but has
received little attention. The analogous reaction between
quinone imides, specifically, N-acetylated quinone imines, has
been investigated with divergent results. 1,2-Fluorenoquinone-
2-acetimide reacted with lysine in serum albumin via 1,4-
addition. In contrast, N-acetyl-3,5-dimethyl-β-benzoquinone
imine reacted with aniline to give the N-phenyl quinone imine,
whereas the 2,6-isomer gave a tetrahedral adduct in which the

Figure 1. (A) HPLC-MS-MS analysis of synthetic 2b (top), isolate from histones in cells treated with 3,5-DMAP (bottom), and isolate from histones in untreated cells (middle). (B) CID spectra of synthetic 2b (top) and 2b isolated from 3,5-DMAP-treated cells (bottom).

Figure 2. HPLC analysis of histones isolated from [14C]3,5-DMAP-
treated cells showing UV detector response (continuous trace) and
[14C] concentration in collected fractions.
acetamido group was retained.\textsuperscript{17} In the present work, we looked for and found no evidence by mass spectrometry for 1,4-addition of N\textsuperscript{\textdagger}-\textit{(t-Boc)}lysine to 3,5-DMQI when forming 2a. 1,4-Addition is clearly not blocked by the methyl substituents, but it is directed toward the unsubstituted positions on the ring: we found that 3,5-DMQI reacts with N-acetyllysine at the 2-position at much greater rates than the rate of transimination with N\textsuperscript{\textdagger}-\textit{(t-Boc)}lysine, and similarly, N-acetyl-3,5-dimethyl-p-benzoquinone imine readily forms a Michael product with ethanethiol.\textsuperscript{17} In both instances, Michael addition takes place relative to the ketone, not the imine.

The biological consequences of quinone imine transimination may be considerable, especially insofar as they relate to hydroxyphenylations of the lysine side chains of the histones. As noted above, this reaction embeds a redox-active center in the nucleosome where it is positioned for maximum efficacy in creating genetic damage via ROS. Additionally, modification (acylation and methylation) of certain lysine side chains is involved in epigenetic change. Their nonbiological modification through hydroxyphenylation may inhibit or replicate these changes inappropriately. The combination of two complementary modes of action may serve to endow certain monocyclic aminophenols with considerably more biological activity than has historically been attributed to them.

\section*{ASSOCIATED CONTENT}

\section*{Supporting Information}
Experimental details and \textsuperscript{1}H NMR and mass spectral data identifying structure 2b. This material is available free of charge via the Internet at http://pubs.acs.org.

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\section*{Notes}
The authors declare no competing financial interest.

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\section*{ABBREVIATIONS}
CID, collision-induced dissociation; DMAP, dimethylaminophenol; DMQL, dimethyl quinone imine; MRM, multiple reaction monitoring; ROS, reactive oxygen species; \textit{t-Boc}, tert-butylxycarbonyl

\section*{REFERENCES}
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(9) This nomenclature differs from the IUPAC system for the purpose of preserving the numbering used for the amines from which the aminophenols and quinone imines are derived.