EFFECT OF SUPEROXIDE DISMUTASE ON THE AUTOXIDATION OF HYDROQUINONES FORMED DURING DT-DIAPHORASE CATALYSIS AND GLUTATHIONE NUCLEOPHILIC ADDITION

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Electron-transfer reactions as well as the generation and reactivity of free radicals in biological systems are controlled by thermodynamic-, kinetic-, and environmental factors. The redox chemistry of hydro- and semi-quinones is in large extent determined by the physico-chemical properties of the molecule, such as the reduction potential and the influence on it of the substitution pattern, by environmental factors, such as pH, solvent cage, solvation energy, and medium polarity, and by kinetic factors, which can allow a reaction -otherwise thermodynamically unlikely- to proceed by exerting a modification on its equilibrium.

The autoxidation of semiquinones -represented by the overall equation: \( Q^- + O_2 \leftrightarrow Q + O_2^- \) is accelerated by superoxide dismutase; pulse radiolysis studies indicated that the equilibrium of the autoxidation reaction is driven towards the right upon removal of \( O_2^- \) by superoxide dismutase. This, along with the reduction of the aminopyrine cation radical by GSH, set examples of kinetically -rather than thermodynamically- controlled reactions.

The effect of superoxide dismutase on hydroquinone autoxidation, on the other hand, is controversial, because the enzyme has been shown -depending on the type of hydroquinone- to inhibit or stimulate \( O_2 \) consumption and \( H_2O_2 \) formation linked to the autoxidation reaction. Superoxide dismutase has been reported to inhibit the autoxidation of trihydroxybenzene, leucoflavins, divicine and dialuric acid, 6-hydroxy-dopamine, dopamine hydroquinone, and pyrogallol.

Recent studies indicated that Cu-Zn superoxide dismutase could either inhibit or enhance hydroquinone autoxidation. Analysis of these effects of superoxide dismutase requires step-wise consideration of the processes involved in the overall hydroquinone autoxidation: [a] the two-electron reduction of the quinone as accomplished during DT-diaphorase catalysis, or GSH nucleophilic addition. [b] One-electron oxidation of the hydroquinone to yield a semiquinone intermediate; this process can involve different redox transi-
tions and the relative contribution of each individual reaction to the overall process will be
determined by thermodynamic-, kinetic-, and environmental factors. [c] One-electron auto­
oxidation of the semiquinone with formation of $O_2^-$. These relationships are illustrated in the
scheme below, in which the state of protonation of the intermediate species is intentionally
ambiguous; likewise, a single process -autoxidation- was used to account for the $Q^- \leftrightarrow Q$
transition, for it is relevant to the further evaluation of the role of superoxide dismutase on
hydroquinone autoxidation.

**Two-electron reduction**

<table>
<thead>
<tr>
<th>DT-diaphorase catalysis</th>
<th>One-electron redox transitions</th>
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<td>1,4-Reductive addition</td>
<td>Auto-oxidation</td>
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<td>Oxidation by superoxide</td>
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<td>Cross-oxidation</td>
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<td>Oxidation by metals</td>
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**Two-electron transfer to quinones: DT-diaphorase catalysis and nucleophilic addition**

Two-electron transfer processes to quinoid compounds are mainly encompassed by
DT-diaphorase and 1,4-reductive addition, e.g., reactions with sulfur nucleophiles such as
GSH. The former activity can be formally understood in terms of a hydride transfer from
the flavoprotein to a two-electron acceptor (reaction 1)

$$
\begin{align*}
R_4 & \quad O \quad R_1 \\
R_3 & \quad O \\
\text{DT-diaphorase catalysis} & \quad \text{Auto-oxidation} \\
\end{align*}
$$

whereas the reaction between quinoid compounds and nucleophiles is a 1,4-reductive addi­
tion of the Michael type (reaction 2). This reaction proceeds with formation of a transition
state anion, which further leads to the generation of a primary thioether sulfide, costumarily
termed -for the case of GSH as sulfur nucleophile- hydroquinone-glutathione conjugate.

$$
\begin{align*}
R_4 & \quad O \quad R_1 \\
R_3 & \quad O \\
\text{(H)} & \quad + \quad \text{GS}^- \quad + \quad \text{H}^+ \\
\text{[2]} & \quad \text{R}_4 \quad \text{OH} \quad \text{R}_1 \\
\text{R}_3 \quad \text{OH} \quad \text{SG}
\end{align*}
$$