

# Sepiapterin Reductase Mediates Redox Cycling and Generates Reactive Oxygen Species

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Several flavin-dependent enzymes have been shown to mediate redox cycling, generating reactive oxygen species (ROS) important to the antimicrobial response, but capable of damaging DNA, lipids, and proteins. Recently, flavin-independent enzymes were discovered, including sepiapterin reductase (SPR), which also mediate redox cycling through unknown mechanisms. We hypothesize that SPR mediates redox cycling and generates ROS— $\text{H}_2\text{O}_2$ ,  $\text{OH}\cdot$ ,  $\text{O}_2\cdot^-$ —here—in the presence of quinones via a one-electron reduction, then reducing oxygen and leading to further ROS generation. Purified recombinant human SPR reduced sepiapterin in the presence of NADPH and when menadione was added, generated ROS whose time and enzyme-dependent rates were measured with  $\text{H}_2\text{O}_2$ ,  $\text{HO}\cdot$ , and  $\text{O}_2\cdot^-$  assays. We found that for all ROS, there exists a linear relationship for both rates ( $0.114 \text{ nmol/min}\cdot\mu\text{g H}_2\text{O}_2$ ,  $2.08 \text{ pmol/min}\cdot\mu\text{g HO}\cdot$ ,  $3.25 \text{ A550 nm} \times 10^{-3}/\text{min}\cdot\mu\text{g O}_2\cdot^-$ ). Formation of  $\text{H}_2\text{O}_2$  increased 119.7% with SOD addition and was reduced  $\geq 100\%$  in the presence of catalase, indicating how the ROS form. With the addition of DPI,  $\text{H}_2\text{O}_2$  formation by SPR was not decreased while thioredoxin reductase activity was. This data confirms that SPR is a key enzyme mediating chemical redox cycling and suggests that it may be important in generating cytotoxic reactive oxygen species in the lung. In contrast to other flavin-reductases, SPR is a flavin-independent reductase mediating redox cycling. SPR-mediated reduction of sepiapterin and redox cycling occur by distinct mechanisms. Taken together, SPR are likely to contribute to lung injury following exposure to dicarbonyls and quinones to some extent. Supported by NIH R25ES020721 and the ASPET SURF Program.

