Oxidation of Thiols by Pyocyanin, A Cytotoxic Product of *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa causes chronic lung infection in patients with cystic fibrosis as well as a severe acute nosocomial pneumonia. Among the virulence factors the organism produces are pyocyanin (1-hydroxy-*N*-methylphenazine) and 1-hydroxyphenazine. Pyocyanin is redox active and stimulates oxidation of NAD(P)H, which in aerobic solutions gives rise to superoxide and hydrogen peroxide through the intermediacy of a pyocyanin radical. We have investigated a number of physiological thiols as possible alternative biological targets for the pigments. We report that thiols (cysteine, glutathione, *N*-acetylcysteine, penicillamine, DTT) react directly with pyocyanin, reducing it to the corresponding radical. In contrast, methionine is inactive. The EPR spectrum of this radical is identical to that generated by reduction of pyocyanin with NAD(P)H. Because reduction of pyocyanin by thiols infers the generation of thiyl radicals, EPR and spin trapping with DMPO was used to verify the formation of these radical species. In addition to thiyl radicals, superoxide was also detected as a DMPO/superoxide adduct from reactions carried out in aerated solutions. In contrast to pyocyanin, 1-hydroxyphenazine appears to be unable to oxidize these thiols, suggesting that the observed biological effects of these two phenazines may be the result of different mechanisms. Because the intracellular concentration of thiols is quite high, they may be preferential targets for pyocyanin. This study identifies thiols as physiologically-important electron donors to pyocyanin. Reactions of pyocyanin with thiols may induce an imbalance in intra/intercellular redox systems, leading to alterations in redox-regulated cell signaling events or direct cytotoxicity.