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Molecular basis of ChrR improvement for metal and radionuclide bioremediation

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Most toxic metals and radionuclides can naturally be precipitated and immobilized by bacterial reduction. This phenomenon has been widely investigated as a promising, inexpensive approach for bioremediation at radioactive waste repository sites. Certain bacteria (e.g. iron- or sulphate-respiring species) have received intense attention as their envelope-located electron transport chain can catalyze such reactions. However, this process is subject to inhibition by nitrate and oxygen, which are usually present at waste sites, and reduced species are released in the extracellular environment, where they may be back-oxidized. In parallel, metal reduction by cytosolic enzymes often involves 1-electron transfer mechanisms that generate reactive oxygen species, which poison the cells and hamper remediation. Our efforts were focused on an alternative "safe" pathway based on the widely distributed ChrR enzyme family of NAD(P)H-dependant FMN-reductases, which catalyze obligatory 2-electron transfers that lessen metal toxicity and increase bacterial remediation capacity with minimum oxidative stress. These reductases contain a non-covalently bound flavin mononucleotide (FMN) as prosthetic group, through which electrons are transferred from NAD(P)H to a broad spectrum of substrates, including metal ions, like chromate and uranyl. Escherichia coli chromate reductase ChrR has been extensively studied for its remediation properties, and various ChrR mutants showing increased metal reduction activity were previously generated, by random mutagenesis or predictions inferred from statistical models. To understand the molecular basis of ChrR improvement and to further improve metal reduction kinetics, we solved the crystal structure of the wild-type protein. The structure analysis revealed that (i) the enzyme smallest functional unit is dimeric, but ChrR proteins can also form tetramers; (ii) amino acid substitutions of improved mutants are not located in the active site of the enzyme (FMN binding domain); (iii) these mutations are mostly confined to the putative surface of interaction through which two dimers form a tetramer. Electrochemical redox titration experiments carried out by UV-visible spectrophotometry showed no significant difference between FMN mid-point potential values of wild-type and improved enzymes, supporting the hypothesis that mutations do not affect FMN interaction with the protein. This finding was further confirmed by Fourier-transform infrared spectroscopy analyses. New mutants were generated to assess the hypothesis that mutations impeding tetramer formation may facilitate substrate and cofactor access to FMN, and a significant improvement of enzymatic activity was indeed observed. Detailed oligomeric properties of ChrR enzymes were thus investigated by gelfiltration, and kinetic measurements are in progress to characterize the enzyme affinity for chromate, uranyl and NAD(P)H. As ChrR improvement consisted of minor mutations, and since potential hazards of using genetically-modified organisms for field remediation remain unknown, we are now seeking for similarly improved enzymes that may have naturally evolved in environments where microorganisms have been living in contact with toxic metals for several decades. While the expected results of this work promise to provide new insights in the development of a novel biological tool for ex-situ bioremediation of metals and radionuclides, it may also allow characterizing indigenous bacteria of which growth and activity stimulation could enhance in situ bioremediation, in a global strategy based on combined chemical and biological approaches.