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Title: ***Spectroscopic characterization of the coproporphyrin ferrochelatase from Corynebacterium diphtheriae***

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Abstract:
The coproporphyrin-dependent heme biosynthesis pathway utilized by monoderm Gram-positive bacteria to produce heme b has been discovered in 2015 [1]. In the penultimate step, the coproporphyrin ferrochelatase (CpfC) catalyzes the insertion of ferrous iron into the coproporphyrin III (cpIII), producing iron coproporphyrin III (coproheme). In the final step, the coproheme decarboxylase generates heme b by a two-step decarboxylation of the propionate groups at positions 2 and 4 of coproheme, forming vinyl groups.
Our group has already investigated the CpfC of the firmicute *Listeria monocytogenes* (*Lm*) [2,3]. Here, we characterized the wild-type (WT) CpfC from actinobacterial *Corynebacterium diphtheriae* (*Cd*CpfC) in its apo form, and complexed with the substrate (cpIII) and the product (coproheme) using UV-vis electronic absorption and resonance Raman (RR) spectroscopies. Unlike the *Lm ferrochelatase*, X-ray diffraction studies of the apo *Cd*CpfC reveal that this bacterial *ferrochelatase* contains a [2Fe-2S] cluster. However, the function of this cluster in this protein is not known, as it does not seem to be involved in the iron insertion process. RR spectroscopy allowed us to obtain information about the structure of the cluster as its stretching modes are sensitive to the type, configuration, symmetry, and nature of the ligands [4].
The spectroscopic characterization of the WT *Cd*CpfC complexed with the substrate (cpIII) and the product (coproheme) indicates that the porphyrin ring, inside the active site, is stabilized by several hydrogen-bond interactions established between polar residues and the propionate groups of the porphyrin ring, as previously observed for the WT and selected variants of CpfC from firmicute *Lm* [2,3].
Moreover, the RR spectra of the CO adducts of the WT and selected variants of *Cd*CpfC complexed with coproheme allowed us to monitor the interactions of the distal polar residues with the iron-bound ligand.

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