Commentary

The Twists and Turns of Enzyme Function

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Abstract. This commentary addresses the recent discovery that CysJ, a component of sulfite reductase, also functions as the electron donor to YcbX, a molybdenum cofactor containing protein involved in the reduction of 6-N-hydroxylaminopurine to adenine.

We largely learn metabolic biochemistry as a series of pathways such as glycolysis, citric acid cycle, histidine biosynthetic pathway, etc. where each step in the pathway is catalyzed by a specific enzyme. Exceptions to this are known and are increasing. In the work by Kozmin et al (12) directed at determining the pathway that allows \textit{Escherichia coli} cells to protect themselves against toxic 6-N-hydroxylaminopurine (HAP) they have demonstrated that only the CysJ component of the sulfite reductase complex (CysJ\textsubscript{4}CysI\textsubscript{4} or \(\alpha_{8}\beta_{4}\)) serves along with YcbX in the reduction of HAP back to non-toxic adenine that can enter back into metabolism via a purine salvaging pathway. Before this work, YcbX was predicted to only be a [Fe\textsubscript{2}-S\textsubscript{2}] cluster-containing protein. Their work not only confirms the function of the \textit{ycbX} open reading frame in \textit{E. coli} but shows that this enzyme also requires the molybdenum cofactor (MoCo) to function. The work thus demonstrates that the CysJ portion of the sulfite reductase complex can have multiple roles in cells by supplying reducing equivalents to other enzymes. This raises the possibility that other examples of redox carrier proteins functioning with multiple acceptor proteins are waiting to be discovered. This observation of multiple functions is not to be confused with so called moonlighting enzymes where more than one function is found in a single polypeptide chain (9) since
here the enzyme is still performing the same function. Another protein implicated in this
detoxification of hydroxylamines is YiiM, which the authors also show to be a MoCo-
dependent enzyme but one that is not reduced by CysJ. Thus the work also indicates the
presence of two previously unknown MoCo dependent enzymes.

**The role of CysJ in sulfite reductase.** To understand how CysJ could function in
the HAP reductase one must consider how it functions in sulfite reductase. Early work
showed that the α-chain on the *E. coli* sulfite reductase (CysJ) contained the NADPH,
FAD, and FMN binding sites and that the β-chain (CysI) contained iron, sulfide and
siroheme. There were indications that the iron in the siroheme was the site at which the
sulfite is bound for reduction. The electron flow was proposed early on to proceed as
follows: NADPH → FAD → FMN → siroheme → sulfite (19). After the crystal
structure of the β-chain CysI was obtained it was clear a [Fe₄-S₄] center was likely used
to transfer the electrons from the flavins of the CysJ through a single cysteine thiol to the
siroheme as shown in Figure 1 (4). Recently the monomeric ferredoxin-dependent
sulfite/nitrite reductase structure was determined (17) and despite the fact that it only has
23% sequence similarity to the *E. coli* enzyme the structure of the enzyme-coenzyme
complexes are identical in both enzymes and both contained the single cysteine thiol
linking the [Fe₄-S₄] cluster to the siroheme (17). In neither of these structures is the
nature of the possible interaction with CysJ revealed since no CysJ is present.

**Likely electron transfer to MoCo and its function in YcbK.** In the role of CysJ
in protecting against N-hydroxylated base analogs the enzyme functions as a partner with
the YcbX molybdoenzyme where it catalyzes the NADPH dependent reduction of its
contained FAD and FMN cofactors and also very likely facilitates electron transfer to the

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MoCo center of the YcbX with the electrons localized at the reduced molybdenum center of the enzyme. These electrons are then used for the reduction of the hydroxylated amine back to the function amine. The essence of this reaction is typical for molybdenum cofactor-containing enzymes that catalyze a net transfer of an oxygen atom from or to a substrate in a two-electron process (7, 8). The atom to which this oxygen is attached can be carbon as in the case of xanthine oxidase, sulfur as in the case of sulfite oxidase and nitrogen as in the case of nitrate reductase. The reaction catalyzed by YcbX is unique because in this reaction a hydroxylamine is reduced to an amine unlike nitrate reductase, where a nitrate group is reduced to a nitrite group. That this transfer is likely occurring through the formation of a protein complex between CysJ and YcbX has been indicated through the work identifying all the protein complexes in E. coli (2).

Nature of the possible interaction between CysJ and YcbX. From what has been presented here and in the Kozmin paper, one could infer that the electron transfer from the FMN of the CysJ directly to the [Mo-S₄] center of the YcbX could be occurring. This type of transfer has never been observed before. However, when one looks at homologs of YcbX, it is clear, due to conserved cysteine residues in their sequences, that they all have the potential to contain multiple [Fe-S] clusters. This being the case then the electrons from the FMN can be transferred through these [Fe-S] cluster(s) on their way to the MoCo. What is really interesting is that the enzyme has had to evolve the ability to donate electrons not only to YcbX but also to CysI and maybe even other enzymes. This aspect of the enzyme is thus like that seen with ferredoxins, thioredoxin and glutaredoxin that can donate electrons to a wide variety of different proteins and is thus not that special (13). Each of these redox carriers is placed in a reduced state by separate oxidoreductase
such as ferridoxin NADPH oxidoreductase and thioredoxin reductase, respectively. The CysJ is then like a thioredoxin reductase and thioredoxin combination where a single enzyme is extracting reducing power from NADPH transferring it to FMN for transfer to the acceptor protein. The redox transfer reactions are also different from these enzymes since they use thiols or [Fe₄-S₄] clusters as their redox carriers. CysJ is different since the flavin is transferring the electrons to the acceptor. Transfer of electrons from flavins to [Fe-S] clusters is well known in many different proteins. An excellent example of this being found in the dihydropyrimidine dehydrogenases where both FAD and FMN transfer electrons in and out of a string of the [Fe₄-S₄] clusters in the same enzyme (16). What is different here is the apparent transfer of electrons from flavins in one enzyme to MoCo in another enzyme through [Fe₂-S₂] centers. The MoCo can be oriented in such a way that it can participated directly in electron-transfer to and from different cofactors (10).

Possible electron flow in the HAP reductase. The native protein is either a α₈β₄ or α₆β₈ (18, 19, 21) and no structure is currently known for the intact complex. As a result we do not know how the flavin in the CysJ maybe located in the CysJ YcbX complex next to the [Fe-S] cluster in YcbX. The flavoprotein component of the enzyme contains two prosthetic groups: one FAD and one FMN binding site (5, 6) each binding site in a different domain that evolved from different proteins (15). The FAD is associated with a ferredoxin-NADP⁺ binding site and the FAD-binding domain is homologous to bacterial flavodoxins. The enzyme belongs to a family of electron transfer flavoproteins that include NADPH-cytochrome P₄₅₀ reductase, nitric oxide synthase, cytochrome P₄₅₀ and methionine reductase.
Source and toxicity of N-hydroxylated compounds. N-hydroxylated base analogs are stated in the paper to be produced by “normal cellular metabolism or by the action of chemical and physical factors, such as alkylating agents or ionizing radiation”. I could not find any examples where this has been shown for HAP production. This work was thus done with a compound that appears to have never been identified as a natural product. The compound can, however, be generated by the microsomal N-hydroxylation of adenine (3) and has been used extensively as a very strong mutagen in bacterial, fungal and mammalian cells (1, 11, 14). Considering the apparent low amount of HAP that may be present in natural systems it is likely that HAP is not the natural substrate for this enzyme.

The manuscript also establishes a possible function for the CysJ-YchX ‘hybrid’ protein encoded by the genomes of two Vibrio species and demonstrated once again that the functional assignment of genomes always require experiments (20).

References


Figure 1. Comparison of electron flow in sulfite dehydrogenase with what may be occurring in the CysJ-YcbX complex. Note that with sulfite reductase, three NADPH are required to reduce one sulfite whereas with HAP reductase only one NADPH is required to reduce one HAP.