Expression of *C. acidovorans* Xanthine Dehydrogenase Containing One FAD and Two [2Fe-2S] Centers but no Mo-pyranopterin Center

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With the demonstration of W-pyranopterin as well as Mo-pyranopterin containing enzymes catalyzing similar reactions in biological systems, it still remains unanswered as how differing enzymes exhibit specificities for each Group VI metal in their respective active sites. In mammalian systems, $[WO_4]^{-2}$ functions as an antagonist for the incorporation of Mo into Mo enzymes.[1,2] It has been shown with sulfite oxidase that a W-pyranopterin is readily incorporated into the enzyme[3] although this metal substitution does not results in any detectable catalytic activity. Other work has shown that $[WO_4]^{-2}$ acts as an antagonist for xanthine oxidase (XO) activity and the basis for this inhibition was attributed to lack of Mo in the enzyme.[4] Previous work in our laboratory on *C. acidovorans* xanthine dehydrogenase (XDH) has shown the enzyme to acquire Mo even in the presence of $[WO_4]^{-2}$. Since the organism required XDH for growth on hypoxanthine, it was difficult to alter conditions to investigate relative antagonistic properties of Mo and W in this system. Our recent development of a high level expression system for *C. acidovorans* XDH in *P. aeruginosa*[5] provides a system without the inherent deficiencies in investigating the W effect on Mo-pyranopterin incorporation. Cells grown in the presence of 0.25 mM $[WO_4]^{-2}$ without added Mo in the medium with IPTG induction provide large quantities of XDH without any detectable catalytic activity. ICP analysis of the purified enzyme shows 4 Fe/$\alpha\beta$ protomer but no detectable W. One mole of FAD/$\alpha\beta$ protomer is also found. CD and low temperature EPR spectra demonstrate the presence of Fe/S I and Fe/S II (both 2-Fe-2S clusters) identical with the native enzyme. The finding that the Mo site is empty accounts for the lack of catalytic activity rather than the formation of an inactive W-pyranopterin-containing enzyme. Metal analyses of whole cells demonstrate their ability to take up $[WO_4]^{-2}$ from the medium so sufficient W is available if it could substitute in the Mo incorporation system. XDH expressed in cells grown in $[WO_4]^{-2}$ then placed in a $[MoO_4]^{-2}$ containing medium without IPTG slowly regain activity. These data show that the machinery for incorporation of the Mo-pyranopterin remains active. Since W has the same diameter and coordination number as Mo, the failure to incorporate W into the enzyme is proposed to be due to an inhibitory property of W on the Mo insertase system. We suggest the low redox potential of W and/or the high affinities of this metal for S and/or O ligands are responsible for this proposed inhibition.

References

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