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Expression and processing of xanthin dehydrogenase/oxidase cDNA from African cape buffalo

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Abstract

Our lab studies oxidative regulation in defense against extracellular pathogens, especially African Trypanosomes. ^ Trypanosomes are protozoan hemoflagellates. The organisms are transmitted by tsetse flies (*Golssina* spp) and cause fatal trypanosomiasis in people and domestic animals. Previous work in our lab showed that African Cape buffalo and eland plasma contain a trypanocidal protein which kills all species of African trypanosomes during short term incubation *in vitro* (Black, 1999). The protein was identified as xanthine oxidase (Muranjan, 1997). On-going work in our lab has shown that the level of xanthine oxidase is a species and breed characteristic. The main focus of this investigation was to explore the molecular basis underlying the intrinsic systemic XO activity differences between different mammal species, with a view toward elucidating strategies that might enhance resistance of domestic animals to trypanosomiasis. ^ My work has shown that the systemic XO activity differences observed in different mammal species, including Cape buffalo, eland, cattle, human, rat and mouse, does not result from XO coding sequence variation. Neither does it result from differences in tissue expression or transcription efficiency. Xanthine oxidase was proposed as a cytosolic protein, lacking any known leader/targeting sequences. My work also showed that there was more intensive signal in the paranuclear region. Expression fragments of the XOR protein revealed unconventional targeting sequence residing in both the N-terminus and the C-terminus of the enzyme, and they all target the recombinant protein to the paranuclear region. Sequence analysis confirmed the presence of two nucleus targeting sequence, which seem to agree with the role of xanthine oxidase in oxidant-mediated signal transduction in ischemia/reperfusion injury. We also found that XOR was not secreted into the medium under standard tissue culture growth conditions, nor was it displayed on the cell surface. This strongly suggests that the XOR protein might not be transported into the circulatory system under normal physiological condition and the presence of XOR in plasma could simply result from its release during cell death as a result of normal cell turn-over. ^

Subject Area

Molecular biology

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