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ORIGINAL PAPER

Nitrite reduction by xanthine oxidase family enzymes: a new class of nitrite reductases

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Abstract Mammalian xanthine oxidase (XO) and *Desulfovibrio gigas* aldehyde oxidoreductase (AOR) are members of the XO family of mononuclear molybdoenzymes that catalyse the oxidative hydroxylation of a wide range of aldehydes and heterocyclic compounds. Much less known is the XO ability to catalyse the nitrite reduction to nitric oxide radical (NO). To assess the competence of other XO family enzymes to catalyse the nitrite reduction and to shed some light onto the molecular mechanism of this reaction, we characterised the anaerobic XO- and AOR-catalysed nitrite reduction. The identification of NO as the reaction product was done with a NO-selective electrode and by electron paramagnetic resonance (EPR) spectroscopy. The steady-state kinetic characterisation corroborated the XO-catalysed nitrite reduction and demonstrated, for the first time, that the prokaryotic AOR does catalyse the nitrite reduction to NO, in the presence of any electron donor to the enzyme, substrate (aldehyde) or not (dithionite). Nitrite binding and reduction was shown by EPR spectroscopy to occur on a reduced molybdenum centre. A molecular mechanism of AOR- and XO-catalysed nitrite reduction is discussed, in which the higher oxidation states of molybdenum seem to be involved in oxygen-atom insertion, whereas the lower oxidation states would favour oxygen-atom abstraction. Our results define a new catalytic performance for AOR—the nitrite reduction—and propose a new class of molybdenum-containing nitrite reductases.

Keywords Nitrite reduction · Nitric oxide formation · Molybdenum · Xanthine oxidase · Aldehyde oxidoreductase

Abbreviations

AOR	Aldehyde oxidoreductase
DMSOR	Dimethylsulfoxide reductase
EPR	Electron paramagnetic resonance
Fe/S	Iron–sulfur centre
Fe/S–NO	Dinitrosyl–iron–sulfur complex
$(MGD)_2$–Fe	Ferrous complex of di(*N*-methyl-D-glucamine dithiocarbamate)
$(MGD)_2$–Fe–NO	Mononitrosyl–iron complex
Mo-enzymes	Pterin–molybdenum-containing enzymes
NaR	Nitrate reductases
NO	Nitric oxide radical
SO	Sulfite oxidase
XO	Xanthine oxidase

Introduction

Molybdenum is present in a wide variety of enzymes, in both prokaryotes and eukaryotes, where it performs cata-