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ORIGINAL PAPER

NADH oxidase activity of rat and human liver xanthine oxidoreductase: potential role in superoxide production

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Abstract To characterise the NADH oxidase activity of both xanthine dehydrogenase (XD) and xanthine oxidase (XO) forms of rat liver xanthine oxidoreductase (XOR) and to evaluate the potential role of this mammalian enzyme as an $O_2^{\bullet-}$ source, kinetics and electron paramagnetic resonance (EPR) spectroscopic studies were performed. A steady-state kinetics study of XD showed that it catalyses NADH oxidation, leading to the formation of one $O_2^{\bullet-}$ molecule and half a H_2O_2 molecule per NADH molecule, at rates 3 times those observed for XO (29.2 ± 1.6 and 9.38 ± 0.31 min^{-1}, respectively). EPR spectra of NADH-reduced XD and XO were qualitatively similar, but they were quantitatively quite different. While NADH efficiently reduced XD, only a great excess of NADH reduced XO. In agreement with reductive titration data, the XD specificity constant for NADH (8.73 ± 1.36 μM^{-1} min^{-1}) was found to be higher than that of the XO specificity constant (1.07 ± 0.09 μM^{-1} min^{-1}). It was confirmed that, for the reducing substrate xanthine, rat liver XD is also a better $O_2^{\bullet-}$ source than XO. These data show that the dehydrogenase form of liver XOR is, thus, intrinsically more efficient at generating $O_2^{\bullet-}$ than the oxidase form, independently of the reducing substrate. Most importantly, for comparative purposes, human liver XO activity towards NADH oxidation was also studied, and the kinetics parameters obtained were found to be very similar to those of the XO form of rat liver XOR, foreseeing potential applications of rat liver XOR as a model of the human liver enzyme.