This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Xanthine Oxidase

by

Ling Li

B180 Medical Laboratories
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2001

8 March, 2001

Abbreviations:
AMP, adenosine monophosphate
ATP, adenosine triphosphate
ESR, electron spin resonance
FAD, flavin adenine dinucleotide
$FADH_2$, fully reduced form of flavin adenine dinucleotide
$FADH^{\bullet}$, neutral semiquinone form of flavin adenine dinucleotide
NAD^+, nicotinamide adenin dinucleotide
XO, xanthine oxidase
XDH, xanthine dehydrogenase

Table of Contents

Abstract

Xanthine oxidase (XO) plays an important role in the catabolism of purines. It has two forms: xanthine oxidase and xanthine dehydrogenase (XDH), and in mammals XDH can be converted to XO form either reversibly or irreversibly. XDH/XO catalyzes the reactions of conversion of hypoxanthine to xanthine, and xanthine to uric acid, transfers electrons to electron acceptors – NAD^+/molecular oxygen, and generates free radicals – hydrogen peroxide/supereoxide. The involvement of this enzyme in ischemia/reperfusion injury has been proposed, but it still remains to be established. This paper briefly reviews some of the properties and reaction mechanisms of XDH/XO.

Introduction

Xanthine oxidase is a highly versatile enzyme that is widely distributed among species (from bacteria to man) and within the various tissues of mammals. It is a member of a group of enzymes known as molybdenum iron-sulfur flavin hydroxylases. It catalyses the hydroxylation of purines, and in particular, xanthine to uric acid [1].

Xanthine oxidase is an important source of oxygen free radicals. The enzyme catalyzes the reduction of O_2, leading to the formation of superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), and it has been proposed as a central mechanism of oxidative injury in some situations [2,3].

Chemical properties

1. Metalloenzyme - Dimer

Xanthine oxidase (EC 1.2.3.2) and xanthine dehydrogenase (XDH, EC 1.1.1.204) are complex metalloflavoproteins that appear to represent alternate forms of the same gene product. The enzymes from various sources are proteins of MW 300,000 Da and are composed of two identical and catalytically independent subunits; each subunit contains one molybdenum center, two iron sulfur centers, and flavin adenine dinucleotide (FAD). Figure 1 shows a structural model of XDH and XO with the three domains [4]. The enzymes consist of around 1,330 amino acids and the amino acid sequence is highly homologous among the rat, mouse, and human enzymes with about 90% identity [4].

2. Conversion of XDH to XO

.

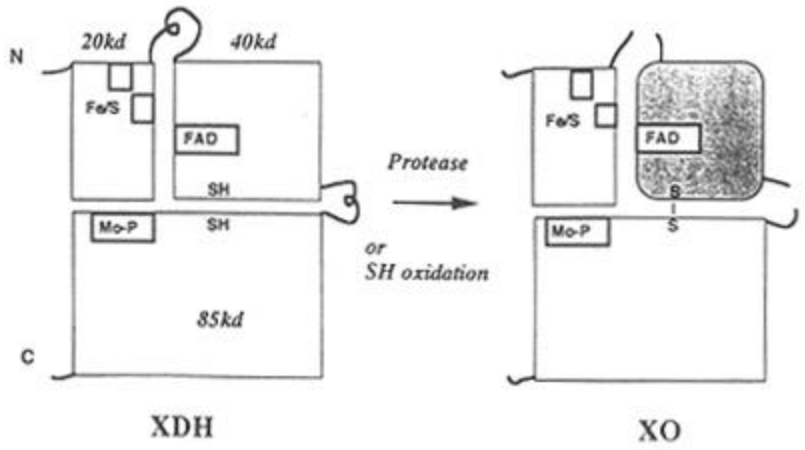

Figure 1. Structural model of xanthine dehydrogenase and oxidase. Although the enzymes consist of dimeric protein, only one subunit is shown. Adapted from [4].

The mammalian enzyme exists originally in the cell as the XDH form but is converted to the XO form by modification of the protein molecule. The conversion occurs either reversibly by oxidation of sulfhydryl residues or irreversibly by proteolysis. The enzyme from rat liver or bovine milk can be isolated without proteolysis as a dehydrogenase when the purification is conducted rapidly or in the presence of thiol reagents. Enzyme isolated in this manner can be reversibly converted to an oxidase by thiol oxidants such as 4,4-dithiopyridine or by limited proteolysis. By using the former procedure it was determined that at least four new disulfide bridges are formed on conversion of the milk dehydrogenase to the oxidase form, although possibly not all are directly involved in the conversion [5]. By limited proteolysis of the mammalian enzyme with trypsin, the enzyme is converted to an XO type with concomitant cleavage into three fragments (20, 40 and 85 kDa). These fragments are only dissociated under denaturation conditions such as in the presence of high concentrations of guanidine-hydrochloride [6]. The most significant difference between the two forms is the protein conformation

around FAD, which changes the redox potential of flavin and the reactitity of FAD with the electron acceptors, NAD^+ and molecular oxygen [4].

3. Electron transfer

The enzyme can accept a total six electrons per subunit from three reducing substrate molecules, two being accepted by the molybdenum, two by the flavin, and one by each of the two iron-sulfur clusters.

$$Mo(VI) \xleftrightarrow{\pm e^-} Mo(V) \xleftrightarrow{\pm e^-} Mo(IV)$$

$$FAD \xleftrightarrow{\pm e^-} FADH \xleftrightarrow{\pm e^-} FADH_2$$

$$2(2Fe(III)2S) \xleftrightarrow{\pm e^-} 2(Fe(II)Fe(III)\text{-}2S)$$

Electron enter the enzyme at Mo(VI), distribute among the 2Fe-2S centers and the flavin, then they leave from the FAD moiety as shown in Figure 2 [4].

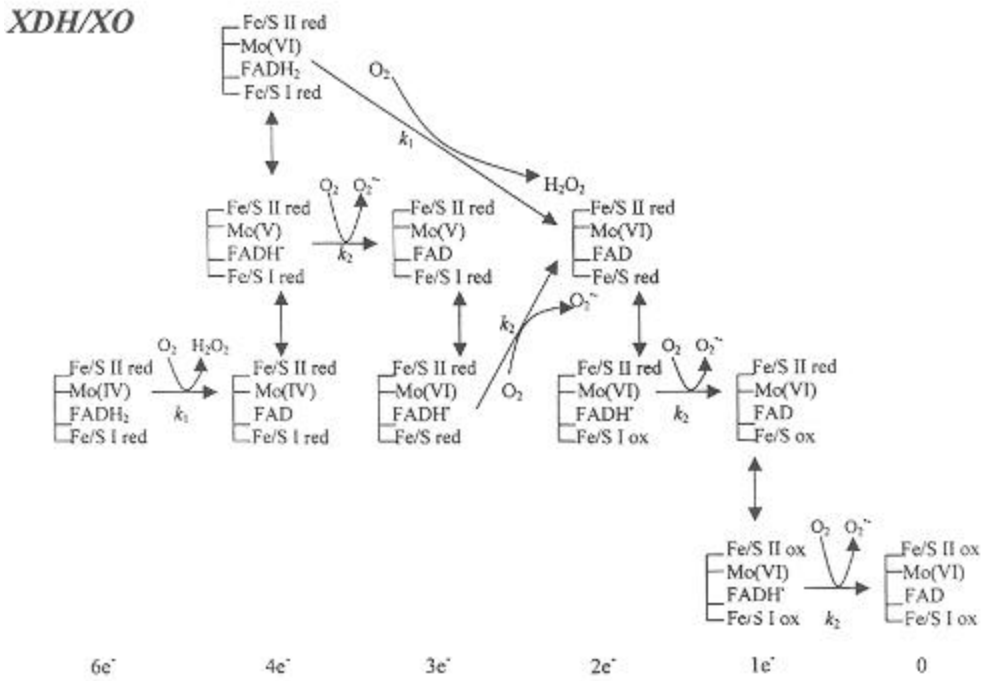

Figure 2. The reaction of XDH with O_2. This scheme has been worked out with chicken XDH because it is not converted to the XO form. $FADH_2$ and $FADH^{\bullet}$ react with oxygen with different rate constants of k_1 and k_2, respectively. Adapted from [4].

Reactions catalyzed by XO

Xanthine oxidase is involved in a major pathway of purine nucleotide and catabolism in animals. The reaction is formally of this type [7]:

$$RH \xrightarrow[-2H^+,\ -2e^-]{+H_2O} ROH \qquad (1)$$

It converts hypoxanthine to xanthine, and xanthine to uric acid.

$$\text{Hypoxanthine} + O_2 + H_2O \xrightarrow{XO} \text{Xanthine} + H_2O_2 + O_2^{\bullet-} \qquad (2)$$

$$\text{Xanthine} + O_2 + H_2O \xrightarrow{XO} \text{Uric acid} + H_2O_2 + O_2^{\bullet-} \qquad (3)$$

A proposed reaction mechanism of xanthine oxidase in the oxidation of xanthine is shown in Figure 3 [8]. The reaction mechanism includes the following steps: (1) the reaction is initiated by attack of enzyme nucleophile, X, on the C3 position of xanthine oxidase; (2) the C8-H atom is eliminated as a hydride ion that combines with the Mo (VI) complex thereby reducing it to the Mo(IV) state; (3) water displaces the enzyme nucleophile producing uric acid.

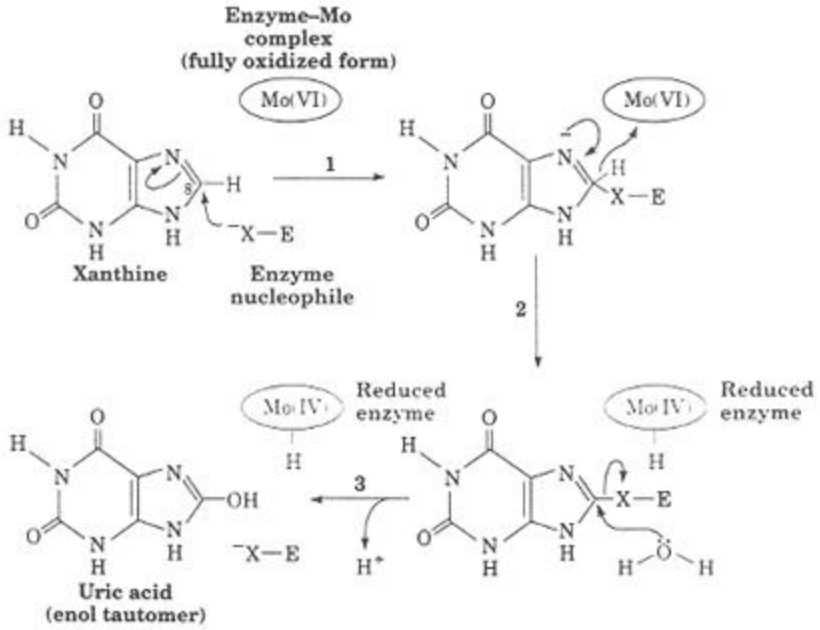

Figure 3. The reaction mechanism of xanthine oxidase in the oxidation of xanthine. Adapted from [8].

Free radicals generated by XDH/XO

Both XDH and XO can form both H_2O_2 and $O_2^{\bullet-}$ when xanthine and molecular oxygen are used as substrates (shown in Figure 2) [4]. The XDH produces more superoxide anion per mole oxygen utilized during turnover than XO does. According to Figure 2, H_2O_2 is produced only from enzyme containing predominantly $FADH_2$, and $O_2^{\bullet-}$ is generated by reaction of O_2 with enzyme where the flavin is in the $FADH^{\bullet}$ oxidation state. The greater amount of formation of $O_2^{\bullet-}$ in XDH is well explained by the greater formation of the flavin semiquinone that reacts with molecular oxygen to form $O_2^{\bullet-}$ [9].

Due to this property, XO can be used as a source of *in vitro* generation of superoxide radicals. It is capable of the univalent reduction of oxygen and of the release of the resultant $O_2^{\bullet-}$ into free solution. It was proposed that the consumption of O_2 by the XO system proceeds by two distinct pathways [10]. These are:

$$\text{Enzyme-}H_2 + 2O_2 \longrightarrow \text{enzyme} + 2H^+ + 2O_2^{\bullet-} \quad (4a)$$

$$2O_2^{\bullet-} + 2H^+ \longrightarrow O_2 + H_2O_2 \quad (4b)$$

$$\text{Enzyme-}H_2 + O_2 \longrightarrow \text{enzyme} + H_2O_2 \quad (5)$$

Figure 4 shows the ESR spectra of $O_2^{\bullet-}$ generated both enzymically and non-enzymically as the evidence for enzymatic reduction of O_2 to $O_2^{\bullet-}$ by XO [11].

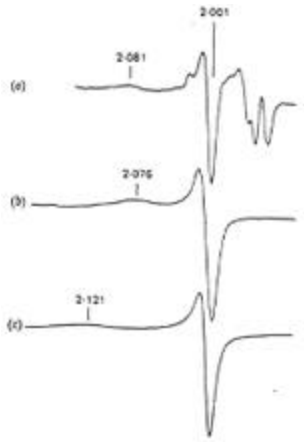

Figure 4. ESR signals ascribed to $O_2^{\bullet-}$ are obtained both enzymically and non-enzymically; the rapid-freezing technique was used for preparing the samples and spectra were recorded at −170 °C. (a) reaction between xanthine and O_2 catalyzed by XO. (b) reaction between H_2O_2 and $NaIO_4$ at pH 9.9. (c) as for (b) but at pH 13.2. Adapted from [11].

The description of the figure is as follows: (a) Reaction between xanthine (2.0 mM) and O_2 (0.76 mM) catalysed by XO (0.20 mM) at pH 10.0 and 23 °C, after a reaction time of 150 ms. Molybdenum signals (overmodulated) are also present, and an iron signal is responsible for the sloping base line. (b) Reaction between H_2O_2 (140 mM) and $NaIO_4$ (70 mM) at pH 9.9 (carbonate buffer) after a reaction time of 600 ms. (c) As for (b) but at pH 13.2 (KOH). For both (b) and (c) a three-syringe system was employed, to enable the peroxide to be made alkaline only shortly before the addition of periodate [10].

XO and ischemia/reperfusion injury

It is thought that XO has an important role in reperfusion injury. One of the mechanisms proposed is that under hypoxic conditions the depletion of the cell's ATP results in an elevated concentration of AMP which is catabolized to adenosine, inosine and then hypoxanthines as shown in Figure 5 [12]. Concomitantly, the conversion of XDH into XO occurs by a protease probably activated by an elevated cytosolic calcium concentration during ischemia. When reperfusion takes place, the return of oxygen leads to a production of superoxide [12].

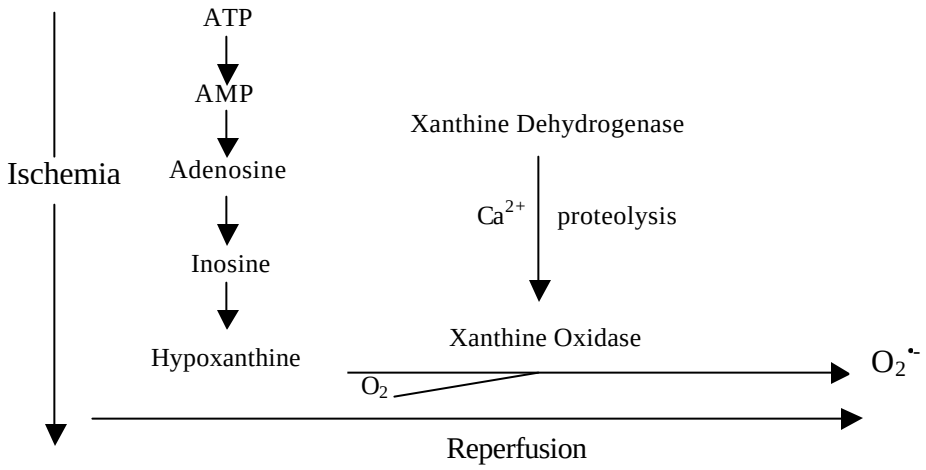

Figure 5. Proposed mechanism of xanthine oxidase mediated free radical injury. Adapted from [12].

The problems remained unclear are that whether the enzymes exist actually in the human organs that may suffer reperfusion injury, and whether XDH converts to XO during ischemia.

Summary

The reaction catalyzed by xanthine oxidase that converts hypoxanthine to xanthine, and xanthine to uric acid is one of the sources of H_2O_2 and $O_2^{\bullet-}$ in biological systems. It is also an important tool in research to generate superoxide. It is proposed that xanthine oxidase may play a role in ischemia/reperfusion injury.

References

1. Massey V, Brumby PE, Komai H. (1969) Studies on milk xanthine oxidase. Some spectral and kinetic properties. *J Biol Chem*. **244**:1682-1691.

2. McCord, JM. (1985) Oxygen-derived free radicals in postischemic tissue injury. *N Engl J Med*. **312**:159-163.

3. Zweier JL, Kuppusamy P, Lutty GA. (1988) Measurement of endothelial cell free radical generation: evidence for a central mechanism of free radical injury in postischemic tissues. *Proc Natl Acad Sci U.S.A.* **85**:4046-4050.

4. Nishino T. (1994) The conversion of xanthine dehydrogenase to xanthine oxidase and the role of the enzyme in reperfusion injury. *J Biochem*. **116**:1-6.

5. Hunt J, Massey V. (1992) Purification and properties of milk xanathine dehydrogenase. *J Biol Chem*. **267**:21479-21485.

6. Amaya, Y, Yamazaki K, Sato M, Noda K, Nishino T, Nishino T. (1990) Proteolytic conversion of xanthine dehydrogenase from the NAD-dependent type to the O_2-dependent type. *J Biol Chem*. **265**:14170-14175.

7. Bray RC, Bennett B, Burke JF, Chovnick A, Doyle WA, Howes BD, Lowe KJ, Richard RL, Turner NA, Ventom A, Whittle JRS. (1996) *Biochemical Society Transaction.* **24**:99-105.

8. Voet D, Voet JG. (1995) *Biochemistry*. 2nd ed. John Wiley & Sons, Inc. pp.816-820.

9. Nishino T, Schopfer LM, Massey V. (1989) The reactivity of chicken liver xanthine dehydrogenase with molecular oxygen. *J Biol Chem*. **263**:13528-13538.

10. Fried R, Fried LW, Babin DR. (1973) Biologic role of xanthine oxidase and tetrazolium reductase inhibitor. *Eur J Biochem*. **33**:439-445.

11. Tubaro E, Lotti B, Santiangeli C, Cavallo G. (1980) Xanthine oxidase: an enzyme playing a role in the killing mechanism of polymorphonuclear leukocytes. *Biochem Parmacol*. **29**:3018-3020.

12. Pasquier C, Pierce B, Willson RL. (1989) Xanthine oxidase mediated free radical injury. In: Hayaishi O, Niki, E, Kondo M, Yoshikawa T eds. *Medical, Biochemical and Chemical aspects of Free Radicals*. pp.425-432.