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Biochimica et Biophysica Acta 1364 (1998) 297–300

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Rapid report

Methylamine dehydrogenase is a light-dependent oxidase

Zhenyu Zhu, Victor L. Davidson *

Department of Biochemistry, The University of Mississippi Medical Center, Jackson, MS 39216-4505, USA

Received 17 February 1998; accepted 19 February 1998

Abstract

Quinoproteins may function either as oxidases or dehydrogenases, depending on the nature of their quinone prosthetic group. The tryptophan tryptophylquinone (TTQ)-linked methylamine dehydrogenase (MADH) is relatively inert towards O_2 in its reduced form. It is shown that on exposure to long range UV (ultaviolet) light, MADH is oxidized in the presence of O_2 and exhibits substrate-dependent steady-state oxidase activity. The effects of light are completely reversible, and oxidase activity is lost when the light is turned off. The light-dependent oxidation of MADH proceeds via a semiquinone intermediate which accumulates to near stoichiometric levels. The absorption of the light appears to provide energy to overcome a thermodynamic barrier to the transfer of an electron from TTQ to O_2. These studies provide a basis for understanding what factors dictate whether an oxidoreductase is a dehydrogenase or an oxidase.

Keywords: Methylamine dehydrogenase; Light-dependent oxidase; Quinoprotein; Tryptophan tryptophylquinone; Ultraviolet; Oxygen

Oxidoreductases may be classified as oxidases or dehydrogenases, depending upon whether or not they use O_2 (oxygen) as an electron acceptor. For flavoproteins and quinoproteins, it is not clear why some function as oxidases and others as dehydrogenases. For example, the FAD-linked mitochondrial acyl-CoA dehydrogenase and peroxisomal acyl-CoA oxidase exhibit significant sequence homology and similar mechanisms for their reductive half-reactions, yet exhibit a 10^6-fold difference in their reactivities towards O_2 [1]. Quinoproteins which possess topaquinone (TPQ) and lysine tyrosylquinone (LTQ) are oxidases, whereas those which possess pyrroloquinoline quinone (PQQ) and tryptophan tryptophylquinone (TTQ) are dehydrogenases [2,3]. TPQ is the cofactor of amine oxidases [4] and TTQ (Fig. 1) is the cofactor of amine dehydrogenases [5] [e.g., methylamine dehydrogenase (MADH)]. These enzymes exhibit essentially identical mechanisms for their reductive half-reactions, and the redox properties of model compounds of TPQ [6] and TTQ [7] are very similar, yet reduced MADH exhibits essentially no reactivity towards O_2. It is important, physiologically, that most dehydrogenases do not react with O_2 because their role is to feed electrons, which are derived from the oxidation of substrates, into the respiratory chain. These enzymes may have evolved to specifically tune the reactivity of the cofactor to

* Corresponding author. Department of Biochemistry, The University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216-4505, USA. Fax: +1-601-984-1501; E-mail: davidson@fiona.umsmed.edu

0005-2728/98/$19.00
PII S0005-2728(98)00035-8

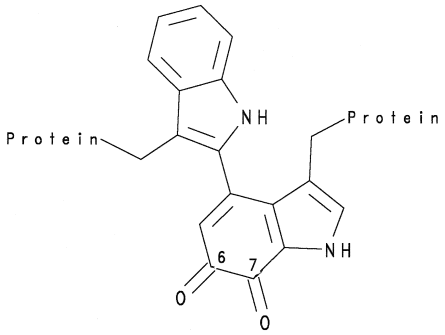

Fig. 1. The structure of oxidized tryptophan tryptophylquinone (TTQ). The C6 and C7 positions are labelled.

prevent reaction with O_2, which would prevent the electrons from being used to drive respiration. We describe here a novel phenomenon, that MADH will exhibit oxidase activity when exposed to long-range UV (ultraviolet) light.

MADH was purified from *Paracoccus denitrificans* (ATCC 13543), as described previously [8]. MADH concentrations were calculated from known extinction coefficients [9] in 10 mM potassium phosphate buffer, pH 7.5. All reagents were obtained from Sigma. Absorption spectra were recorded with a Milton Roy Spectronic 3000 Array spectrophotometer. In oxygen consumption experiments, the O_2 concentration was monitored with a Clark-type oxygen electrode. The source for long-range UV light was a Rad-Free RF UV-365 Long-Wave UV Lamp (Schleicher & Schleicher). It uses an 8-W bulb that emits light which spans the wavelength from 320–380 nm with a peak value at 365 nm. The UV light source was placed approximately 3 in. from the sample, which was contained in a quartz cuvette. The source of strong visible light was a 40-W halogen lamp, which was positioned next to the sample. Room light describes the work place which is illuminated by fluorescent lights and sunlight from windows.

MADH may be reduced by chemical reductants, such as dithionite, or by its substrate, methylamine [9]. Dithionite reduction yields a quinol form of the enzyme, while substrate generates an aminoquinol, in which the C6 quinone oxygen of the TTQ cofactor is replaced by a substrate-derived amino group [10]. It has been generally believed that these reduced forms of MADH are extremely inert towards O_2 and only oxidize very slowly under aerobic conditions. We initially observed that the rate of this very slow oxidation was dependent on exposure to room light, and further investigated this phenomenon. In the dark, reduced MADH is essentially inert towards O_2. Exposure to a strong source of visible light had no effect on the reactivity of reduced MADH towards O_2. However, when reduced MADH was exposed to a source of long-range UV light, a substantial increase in the rate of oxidation of the reduced MADH was observed (Table 1). The dependence of the reaction on long-range UV light, rather than visible light, is consistent with the absorption spectrum of reduced MADH which exhibits a maximum at 330 nm. The oxidation rate was dependent both on the concentration of O_2 and the intensity of the light. As the distance from the light source was increased, the rate of oxidation decreased markedly. If the concentration of O_2 was decreased, the rate also decreased. Thus, the observed rates of light-dependent oxidation listed in Table 1 are not the maximum possible, and would likely increase further on irradiation with a stronger light source. Analysis of the light-dependent spectral changes indicated that the oxidation of reduced MADH proceeded through a readily detectable semiquinone intermediate (Fig. 2).

To determine whether the light-dependent oxidation of MADH was causing any irreversible modification or inactivation of the enzyme or cofactor, or whether O_2 was able to function as a viable electron acceptor, the steady-state oxidase activity of MADH was examined. In the dark, or in the presence of strong visible light, no O_2 consumption was detected. In the presence of long-range UV light, substrate-dependent O_2 consumption was readily observed (Fig. 3). Continuous exposure to the light is required. Oxidase activity stops when the light source is turned off, and begins again when the light is turned back on

Table 1
Light-dependent oxidation of reduced MADH

Light conditions	Half-life of reduced MADH (s)
Dark	$\gg 10^5$
Strong visible light	$\gg 10^5$
Room light	1.3×10^4
Long-range UV light	34

Light sources are described under experimental procedures.

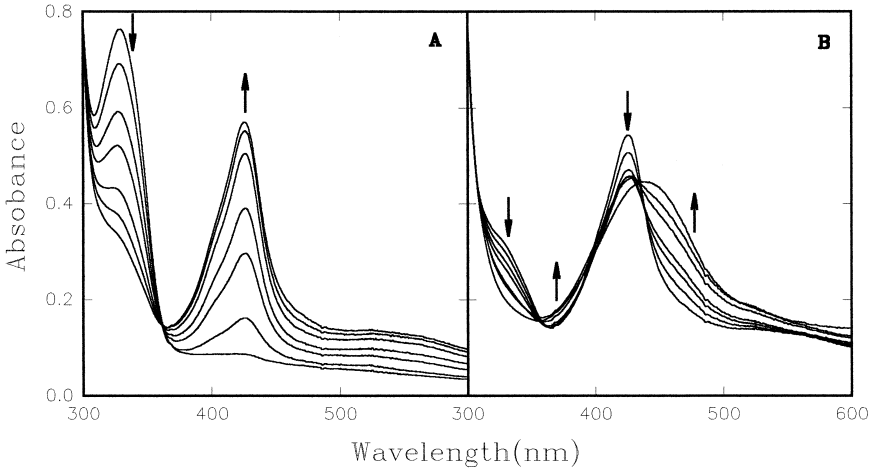

Fig. 2. Light-dependent oxidation of MADH. The reduced form of MADH in this figure was generated by stoichiometric titration with sodium dithionite. This sample contains 15 μM reduced MADH in 10 mM potassium phosphate, pH 7.5, at 25°C. To initiate the reaction, a long-range UV light source was placed directly over the cuvette, and spectra were recorded with time. Panel A displays the early stage of the reaction in which nearly stoichiometric formation of the semiquinone form of MADH is achieved. These spectra were recorded at $t = 0$, 36, 56, 75, 94, 184 and 689 s. Panel B displays the remainder of the reaction in which the semiquinone intermediate is converted to the fully oxidized form. These spectra were recorded at $t = 25$, 36, 45, 49, 64 and 127 min. The direction of the spectral changes are indicated with arrows.

(Fig. 3). Thus, it is clear that while exposed to long-range UV light, this dehydrogenase is able to function as an oxidase.

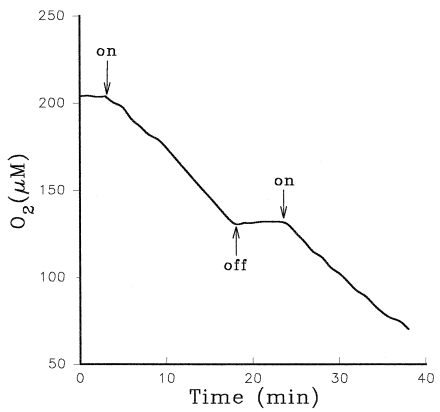

Fig. 3. Light- and substrate-dependent steady-state O_2 consumption by MADH. The reaction mixture contained 9 mM MADH and 200 mM methylamine in 10 mM potassium phosphate, pH 7.5, at 25°C. The O_2 concentration was monitored with a Clark-type oxygen electrode. A long-range UV light source was placed directly next to the quartz vessel which contained the reaction mixture. The times at which the light was turned on and off are indicated. In control experiments with buffer alone, buffer plus MADH, and buffer plus methylamine, no O_2 consumption was observed.

With dehydrogenases, the oxidation of the reduced enzyme by O_2 is typically extremely slow, and the products of the oxidation reaction are usually poorly defined. The reasons for this poor reactivity with oxygen are not understood, and pose an interesting challenge to the field of enzymology. During the reaction mechanism of flavoprotein oxidases, the reduced flavin reacts with O_2 to form flavin semiquinone and superoxide anion, which then are believed to combine to form a covalent flavin hydroperoxide intermediate [11]. Conversely, in flavin electron transferases, such as flavodoxin, the radical pair dissociates. This is thought to be due, at least in part, to the inaccessibility of the C(4a) position of the flavin which is involved in flavin hydroperoxide formation in the oxidases. When MADH is exposed to long-range UV light, which is coincident with the absorption maximum of its reduced form of 330 nm (Fig. 2), its reactivity with O_2 is increased by several orders of magnitude (Table 1). The energy of that incident light is likely being used to overcome a thermodynamic barrier to the transfer of an electron from fully reduced TTQ to O_2. The redox potential for the O_2/O_2^- couple is -330 mV in water [12]. This is much lower than that of the MADH semiquinone/reduced couple of $+190$ mV [13]. Thus, in the absence of light, the one-electron reduc-

tion of O_2 is likely to be very unfavorable, and any superoxide formed would be expected to return an electron to the semiquinone.

Several aromatic compounds are photosensitized, and their irradiation in the presence of O_2 may cause self-sensitized photooxidation [14]. Such a similar phenomenon has not been reported for an enzyme-bound organic cofactor. During the light-dependent oxidation of MADH, there are no small molecules present to act as sensitizing agents. It is likely that reduced TTQ is sensitized by the UV light and reacts with O_2 to form the TTQ semiquinone and superoxide anion. Hydroperoxide adduct formation with TTQ, as seen with the flavoprotein oxidases, is unlikely, since only the C6 carbon of TTQ is exposed to solvent in the active site [15,16], and in the semiquinone state, this would not be a reactive electrophilic site. Furthermore, the active site of MADH resides at the end of an aqueous channel [15,16], which may facilitate the diffusion of superoxide from the active site. This suggests that with MADH, the basis for its normal function as a dehydrogenase, rather than an oxidase, is both thermodynamic and structural. The light-induced alteration in reactivity towards O_2 cannot be attributed to changes in O_2 accessibility, structural changes in the enzyme, or changes in the active site environment due to ligand binding, as was suggested for the flavoproteins, acyl-CoA dehydrogenase [1] and lactate oxidase [17].

Some quinoproteins are oxidases. It is interesting to note that TPQ-dependent amine oxidases possess tightly bound copper in close proximity to TPQ (2). It has been proposed that after reduction of the TPQ cofactor, a Cu(I)/TPQ$\cdot$ species forms, which then reacts with O_2 [18]. Thus, for quinoprotein oxidases, copper may be required to activate the reduced quinone cofactor for electron transfer to O_2. Quinoprotein dehydrogenases lack copper. With MADH, it appears that light may serve a similar role of generating a form of the cofactor which is activated for reaction with O_2. It remains to be seen how common a phenomenon this may be. Enzymologists have not routinely examined enzyme activity as a function of light, wavelength or intensity. Further studies will lead to a better understanding of the energetics of this activation process, which can then be compared with the well-characterized energetics of the physiologic electron transfer reaction from MADH to the type I copper protein, amicyanin, its natural electron acceptor [19,20].

This work was supported by NIH grant GM-41574.

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