Volume 279, number 1, 30-32 FEBS 09369 February 1991

ADONIS 001457939100396

Triiodide reduction by cellobiose:quinone oxidoreductase of *Phanerochaete chrysosporium*

Wenjun Bao and V. Renganathan

Department of Chemical and Biological Sciences, Oregon Graduate Institute of Science and Technology, 19600 NW Von Neumann Drive, Beaverton, OR 97006-1999, USA

Received 22 October 1990

Cellobiose:quinone oxidoreductase (CBQase) in the presence of cellobiose inhibits peroxidase-catalyzed oxidation of iodide to triiodide (I_3^-). This inhibition is due to the two-electron reduction of I_3^- by CBQase. The apparent K_m of I_3^- for this reaction is 120 μM and the specific activity is 57 $\mu mol \cdot min^{-1} \cdot mg^{-1}$. A proposed mechanism for I_3^- reduction by CBQase involves initial reduction of the flavin moiety by cellobiose to produce a dihydroflavin. This is followed by the substitution of one of the iodine atoms of I_3^- at the C(4a)-position of dihydroflavin to generate C(4a)-iododihydroflavin and two iodide ions. The C(4a)-iododihydroflavin eliminates HI to regenerate the oxidized CBQase.

Cellobiose:quinone oxidoreductase; Cellobiose; Triiodide; Peroxidase; Flavodehydrogenase; *Phanerochaete chrysosporium*

1. INTRODUCTION

White-rot basidiomycetous fungi are the only organisms known to be capable of degrading both lignin and cellulose to CO_2 and H_2O [1,2]. Cellulose-degrading cultures of the white-rot fungus *Phanerochaete chrysosporium* produce two extracellular oxidative enzymes, cellobiose:quinone oxidoreductase (CBQase) and cellobiose oxidase, in addition to cellulases [3,4]. Both enzymes oxidize cellobiose to cellobionolactone. CBQase is a flavoenzyme and requires a quinone for activity [3], whereas cellobiose oxidase is a hemoflavoenzyme and apparently requires O_2 as a substrate [4]. CBQase reduces quinones to hydroquinones in the presence of cellobiose [3]. Ligninolytic cultures of *P. chrysosporium* produce two extracellular peroxidases, lignin peroxidase (LiP) and manganese peroxidase (MnP) [1,2,5-7]. CBQase, in the presence of cellobiose, inhibits peroxidase-catalyzed decarboxylation of vanillic acid and LiP-catalyzed oxidation of 3,4-dimethoxybenzyl alcohol and polymerization of kraft lignin [8]. Ander et al. have proposed that CBQase inhibits these reactions through the reduction of phenoxy and aromatic cation radical intermediates [8]. However, Odier et al. have suggested that CBQase does not reduce phenoxy radical intermediates [9]. The mechanism of CBQase inhibition of peroxidase reactions requires further study.

Peroxidase reactions involve initial oxidation of the native enzyme by H_2O_2 to produce compound I which has two oxidizing equivalents more than the native enzyme [10]. Donation of an electron from a substrate such as a phenol, reduces compound I to II, and the addition of a second electron to compound II regenerates the native peroxidase [10]. CBQase inhibition of peroxidase reactions requires cellobiose, and this suggests the involvement of reduced CBQase [8]. Electron transfer from the reduced CBQase to peroxidase compound I and II would also inhibit peroxidase reactions because the peroxidase intermediates would not be available for substrate oxidation. Such a mechanism will inhibit all peroxidase reactions including the reactions that do not produce radical intermediates. To test this suggestion, we studied the effect of CBQase on the peroxidase-catalyzed oxidation of iodide to I_3^-, which apparently involves only ionic rather than radical intermediates [11]. CBQase, in the presence of cellobiose inhibited iodide oxidation. However, this inhibition appears to be due to the two-electron reduction of I_3^- by CBQase and not due to the reduction peroxidase compound I and II.

Correspondence address: V. Renganathan, Department of Chemical and Biological Sciences, Oregon Graduate Institute of Science and Technology, 19600 NW Von Neumann Drive, Beaverton, OR 97006-1999, USA

Abbreviations: ABTS, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); CBQase, cellobiose:quinone oxidoreductase; DCPIP, 2,6-dichlorophenol-indophenol; FPLC, fast protein liquid chromatography; HRP, horseradish peroxidase; LiP, lignin peroxidase; MnP, manganese peroxidase

2. MATERIALS AND METHODS

2.1 *CBQase*

P. chrysosporium strain OGC101 was grown with cotton linters (10

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$g \cdot l^{-1}$) in agitated cultures as described previously [12]. CBQase was purified to homogeneity from the extracellular medium by a procedure involving ammonium sulfate precipitation, DEAE-Sephadex, phenyl Sepharose, Sephacryl S-200 and Mono-Q (FPLC) chromatographic procedures (Bao and Renganathan, unpublished results). Specific activity of CBQase with dichlorophenol-indophenol (DCPIP) as an electron acceptor was 8 $U \cdot mg^{-1}$.

2.2 *Peroxidases*

LiP and MnP were purified from the extracellular medium of lignin-degrading cultures of *P. chrysosporium* as described previously [6,7,13,14]. HRP type VI was purchased from Sigma Chemical Co. (St. Louis, MO)

2.3 *Enzyme assays*

CBQase was assayed with cellobiose (100 μM) and DCPIP (25 μM) at 515 nm ($\epsilon = 6.8\ mM^{-1} \cdot cm^{-1}$), in 20 mM succinate buffer, pH 4.5 [12]. LiP activity was determined with 3,4-dimethoxybenzyl alcohol and H_2O_2, at 310 nm [5,6]. MnP activity was assayed with 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and H_2O_2, at 415 nm in 20 mM lactate, pH 4.5 [7].

2.4. *Iodide oxidation and triiodide reduction*

Peroxidase-catalyzed oxidation of iodide, and CBQase-catalyzed reduction of I_3^- were monitored by following the absorbance changes at 355 nm [11]. Peroxidase assays contained iodide (1 mM), H_2O_2 (100 μM) and cellobiose (100 μM), when required. CBQase assays contained cellobiose (100 μM), I_3^- (50 μM), and iodide (950 μM). All the reactions were performed in 20 mM succinate buffer, pH 4.5. Triiodide solutions were prepared by dissolving iodine in potassium iodide solution with the aid of ultrasonication (30-120 s).

Kinetics of CBQase reduction of I_3^- were performed at a fixed concentration of cellobiose (100 μM) and iodine (50 μM) and at varying concentrations of I_3^- solution (5 μM iodine in 2.5 M iodide solution). K_m and V_{max} were determined from double reciprocal plots of the substrate concentration (I_3^-) versus the initial velocity.

3. RESULTS AND DISCUSSION

CBQase is a flavin-dependent dehydrogenase present in the extracellular medium of cellulose-degrading cultures of the white-rot basidiomycete *P. chrysosporium* [3]. CBQase oxidizes cellobiose to cellobionolactone in the presence of electron acceptors such as quinones and DCPIP [3,12]. Peroxidases such as HRP, LiP, and MnP oxidize iodide to I_3^- in the presence of H_2O_2 ([11,15], Valli and Gold, unpublished results). In this reaction, iodide reduces compound I by two electrons to produce the native peroxidase and hypoiodous acid or an iodonium ion ($HOI \rightleftharpoons OH^- + I^+$) [11]. The latter complexes with two equivalents of iodide to yield I_3^-. CBQase, in the presence of cellobiose, inhibited the LiP-, MnP-, and HRP-catalyzed oxidation of iodide (Fig. 1). The initial velocity and the total I_3^- formed in the reaction decreased. CBQase or cellobiose alone did not cause any inhibition. The level of inhibition was dependent on both CBQase and cellobiose. CBQase, in the presence of cellobiose, also reduced chemically prepared I_3^-. The pH optimum for this reduction was 4.5, which is similar to that of the CBQase reaction with quinone or DCPIP as electron acceptors [3,12]. The ratio of cellobiose oxidized to I_3^- reduced was 1:0.8. These findings suggest that CBQase inhibits peroxidase-catalyzed iodide oxidation by reducing I_3^- by two electrons ($I_3^- + 2e^- \rightarrow 3I^-$). The reducing equivalents are obtained by oxidizing cellobiose. The apparent K_m of I_3^- for this reaction was 120 μM, and the specific activity for I_3^- reduction was 57 $\mu mol \cdot min^{-1} \cdot mg^{-1}$.

I_3^- is an oxidant and the redox potential for two-electron reduction of I_3^- is 0.54 V [16]. Two-electron reduction of I_3^- yields three iodide ions ($I_3^- + 2e^- \rightleftharpoons 3I^-$). The redox potentials of flavoenzymes range from -0.45 V to $+0.15$ V [17]. Since I_3^- is at a higher potential compared to the flavin, electron transfer from the reduced flavin to I_3^- will be favored. Fig. 2 presents a possible mechanism for I_3^- reduction by CBQase. It involves substitution of one of the iodine atoms of I_3^- at the C(4a)-position of the reduced flavin and elimination of the other two iodine atoms as iodide ions. The C(4a)-iododihydroflavin intermediate is

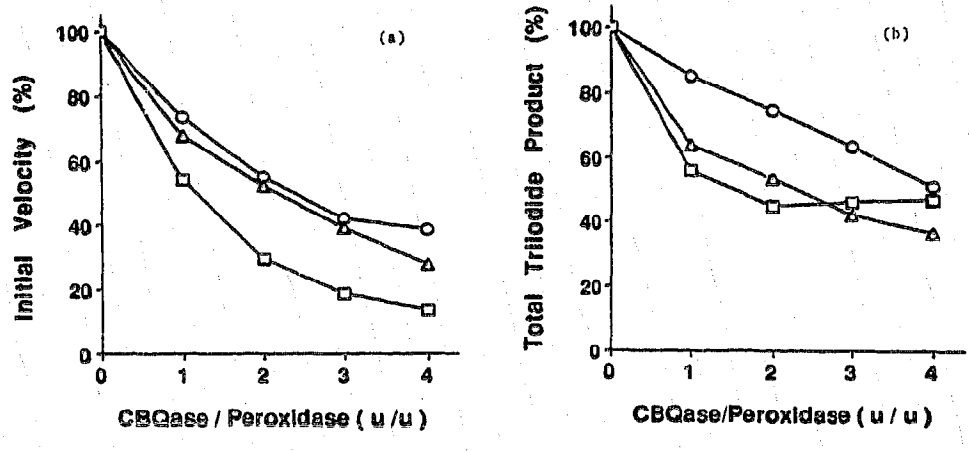

Fig. 1. CBQase inhibition of peroxidase catalyzed oxidation of iodide to triiodide (I_3^-) in the presence of cellobiose: (a) Inhibition of initial velocity; (b) Decrease in total I_3^- product formed. One hundred percent initial velocity and one hundred percent total triiodide product refer to the peroxidase activity observed in the absence of CBQase and cellobiose. Symbols: HRP (○), MnP (△), and LiP (□).

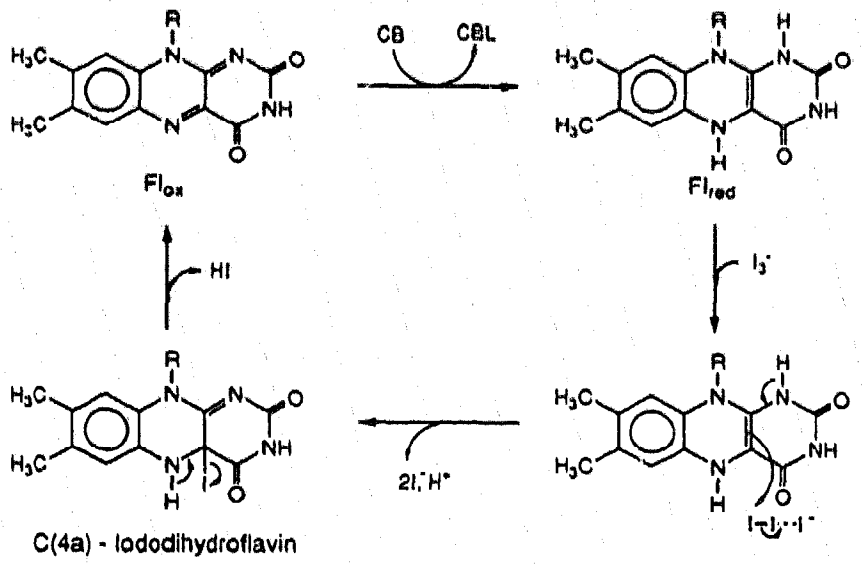

Fig. 2. A probable mechanism of triiodide (I_3^-) reduction by CBQase in the presence of cellobiose. Fl_{ox}, oxidized flavin; Fl_{red}, reduced flavin; CB, cellobiose; CBL, cellobionolactone.

structurally similar to C(4a)-hydroxyflavin, proposed as an intermediate in the flavin-dependent monooxygenase reaction [17]. C(4a)-Hydroxyflavin eliminates a molecule of water to regenerate the oxidized flavin. Similar elimination of HI from C(4a)-iododihydroflavin will yield the oxidized flavin (Fig. 2). Two other flavin-dependent enzymes, glucose oxidase and diaphorase (lipoyl dehydrogenase) were tested for their ability to reduce I_3^-. Glucose oxidase, in the presence of glucose, did not reduce I_3^- either under aerobic or anaerobic conditions. Since NAD(P)H reduced I_3^-, the ability of diaphorase to reduce I_3^- could not be determined.

Acknowledgements: This work was supported by a grant from the US Department of Energy, Office of Basic Energy Sciences (DE-FG06-87ER 13715). The authors wish to thank M.H. Gold, K. Valli, and H. Wariishi for their critical reading of this manuscript. Special thanks to M Stankovich of the University of Minnesota for useful discussions.

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