Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1604 (2003) 47–54
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

The heme domain of cellobiose oxidoreductase: a one-electron reducing system

Maria G. Mason[a], Peter Nicholls[a,*], Christina Divne[b], B. Martin Hallberg[b], Gunnar Henriksson[c], Michael T. Wilson[a]

[a] *Department of Biological Sciences, University of Essex, Central Campus, Wivenhoe Park, Colchester, Essex CO4 3SQ, UK*
[b] *Department of Biotechnology, Division of Wood Biotechnology SCFAB, Royal Institute of Technology (KTH), Stockholm SE-106 91, Sweden*
[c] *Department of Pulp and Paper Chemistry and Technology, Royal Institute of Technology (KTH), Stockholm SE-100 44, Sweden*

Received 7 June 2002; received in revised form 24 February 2003; accepted 26 February 2003

Abstract

Phanerochaete chrysosporium cellobiose oxidoreductase (CBOR) comprises two redox domains, one containing flavin adenine dinucleotide (FAD) and the other protoheme. It reduces both two-electron acceptors, including molecular oxygen, and one-electron acceptors, including transition metal complexes and cytochrome *c*. If the latter reacts with the flavin, the reduced heme *b* acts merely as a redox buffer, but if with the *b* heme, enzyme action involves a true electron transfer chain. Intact CBOR fully reduced with cellobiose, CBOR partially reduced by ascorbate, and isolated ascorbate-reduced heme domain, all transfer electrons at similar rates to cytochrome *c*. Reduction of cationic one-electron acceptors via the heme group supports an electron transfer chain model. Analogous reactions with natural one-electron acceptors can promote Fenton chemistry, which may explain evolutionary retention of the heme domain and the enzyme's unique character among secreted sugar dehydrogenases.

Keywords: Cellobiose; Cellobiose dehydrogenase; Heme domain; Ascorbate; Cellobiose oxidoreductase; Cytochrome *c*; Heme *b*; Electron transfer; Lignin; *Phanerochaete chrysosporium*

1. Introduction

Cellobiose oxidoreductase (cellobiose dehydrogenase, E.C.C. 1.1.99.18, CBOR, CDH) is secreted by the white rot fungus *Phanerochaete chrysosporium*, during lignocellulose degradation [1]. Other fungi secrete external glucose oxidase flavoenzymes [2,3]. CBOR (CDH) is unique in comprising two redox domains, one containing a flavin (FAD) and the other a protoheme group. It transfers reducing equivalents from cellobiose to two types of redox acceptor: (a) two-electron oxidants, including redox dyes, benzoquinones [1] and molecular oxygen; and (b) one-electron oxidants, including semiquinone species, ferric iron complexes, and the model acceptor cytochrome *c* [4,5].

Wilson and Liu [4] and Rogers et al. [5] have argued that while two-electron events involve interaction of reduced flavin and acceptor, one-electron processes first require reduction of heme and then reduction of acceptor by the heme. This model is one of a simple electron transfer chain. In an alternative model, Henriksson et al. [6,7], supported by Cameron and Aust [8], and also in part by Samejima and Eriksson [9], proposed that both one-electron and two-electron acceptors react with the flavin domain, the heme acting solely as a "sink" for the extra electron when one-electron acceptors oxidize the flavin. One-electron acceptors may thus either react preferentially with the flavin, in the "sink" model, or always with the heme *b*, in the "chain" model.

Limited proteolysis of intact enzyme creates separate heme and flavin domains [6]. X-ray crystal structure determination for the CBOR heme domain [10] has demonstrated a unique heme-binding β-sheet tertiary structure and also considerable solvent exposure of the bound protoheme. It has not immediately clarified the problem of heme group

Abbreviations: CBOR, cellobiose oxidoreductase (holoenzyme); CDH, cellobiose dehydrogenase (holoenzyme or flavin domain); FAD, flavin adenine dinucleotide; DCPIP, dichlorophenol indophenol

* Corresponding author. Tel.: +44-1206-873-776/333; fax: +44-1206-872592.
E-mail address: pnicholl@essex.ac.uk (P. Nicholls).

0005-2728/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0005-2728(03)00023-9

reactivity, but homogeneous samples of isolated heme domain permitted the present study.

Experiments described here show that the heme domain, reduced by ascorbate, can transfer electrons rapidly to cytochrome *c*. The reduction rate is similar to that with the intact enzyme, when the latter is reduced either by ascorbate or by cellobiose. Obligatory involvement of the flavin domain in such reactions can therefore probably be

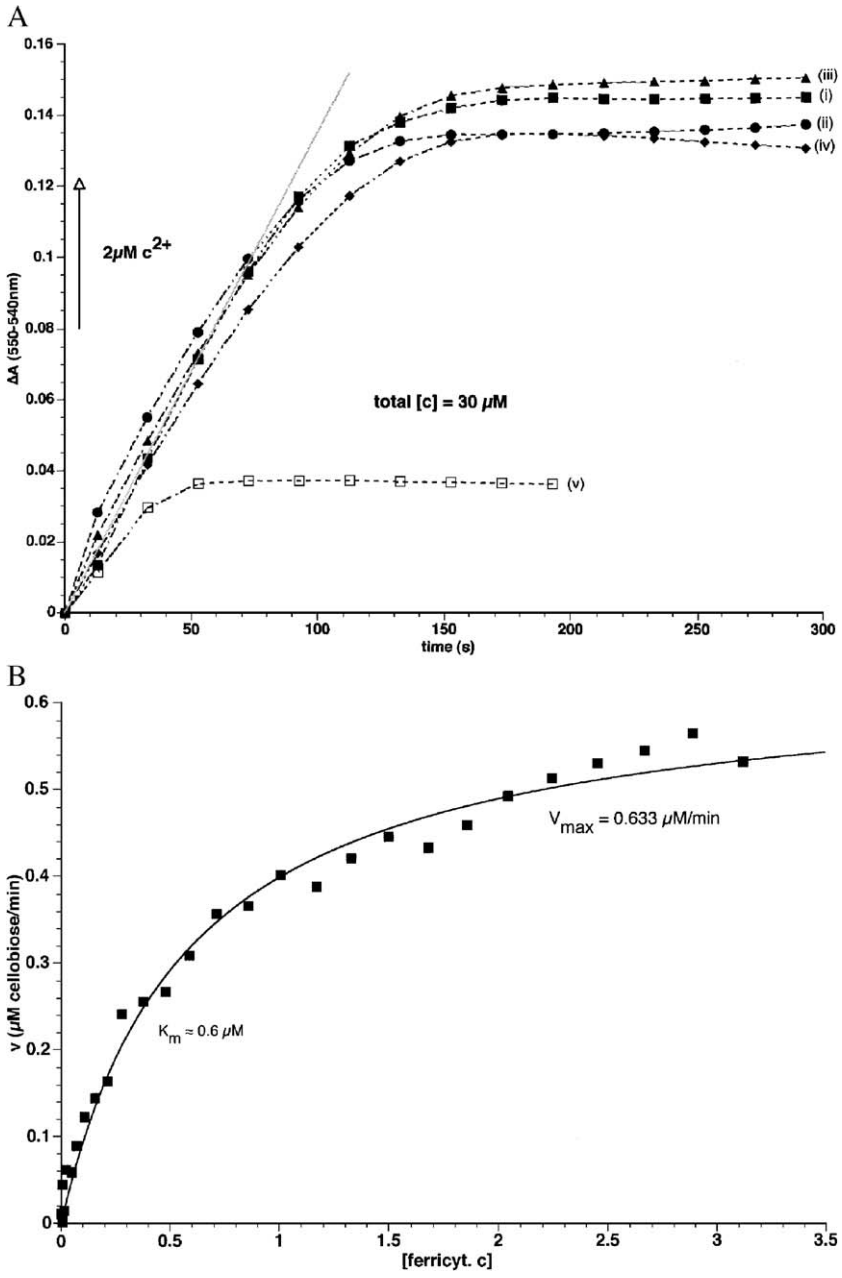


Fig. 1. Reduction of cytochrome *c* by fully (cellobiose) reduced CBOR or by partially (ascorbate) reduced CBOR. (A) Time courses of the overall reaction with cellobiose as reductant substrate at varying [ferricytochrome *c*] levels and a constant total [cytochrome *c*]. Successive additions of 4 μM cellobiose were made to a solution of 30 μM cytochrome *c*, 50 mM potassium phosphate, 100 μM DTPA, pH 6.0, 25 °C, 200 nM CBOR; measurement at 550–540 nm; 3-ml total volume; 1-cm light path; starting redox values: (i) (■) 30 μM ferricyt. *c* (0% reduced); (ii) (●) 22.5 μM ferricyt. *c* (25% reduced); (iii) (▲) 15 μM ferricyt. *c* (49% reduced); (iv) (♦) 8 μM ferricyt. *c* (74% reduced); (v) (□)1.5 μM ferricyt. *c* (95% reduced). (B) Rate of cytochrome *c* reduction vs. ferricytochrome *c* concentration: data obtained during a continuous overall reaction; 8.8 μM cellobiose added initially; 3-ml total volume; 1-cm light path; 10 μM total cytochrome *c*, 14 nM CBOR, pH 5.5 50 mM K phosphate buffer 25 °C; reduction of cytochrome *c* measured at 550–540 nm. (C) Oxidation of 1.7 μM (final) reduced intact CBOR by 6 μM (final) cytochrome *c* in pH 6.0, 0.1 M potassium phosphate buffer 22 °C. CBOR was reduced with 40 mM ascorbate in one syringe. Static spectrophotometry confirmed that only the heme groups are reduced under these conditions. Stopped flow apparatus was used in the diode array mode. Traces fitted with Deltagraph™ 4.0 for Macintosh. The continuous traces represent a single exponential with a first order rate constant of 23.3 s^{-1}. Upper data (●): cytochrome *c* reduction (548.6 nm). Lower data (■): heme *b* oxidation (561.8 nm).

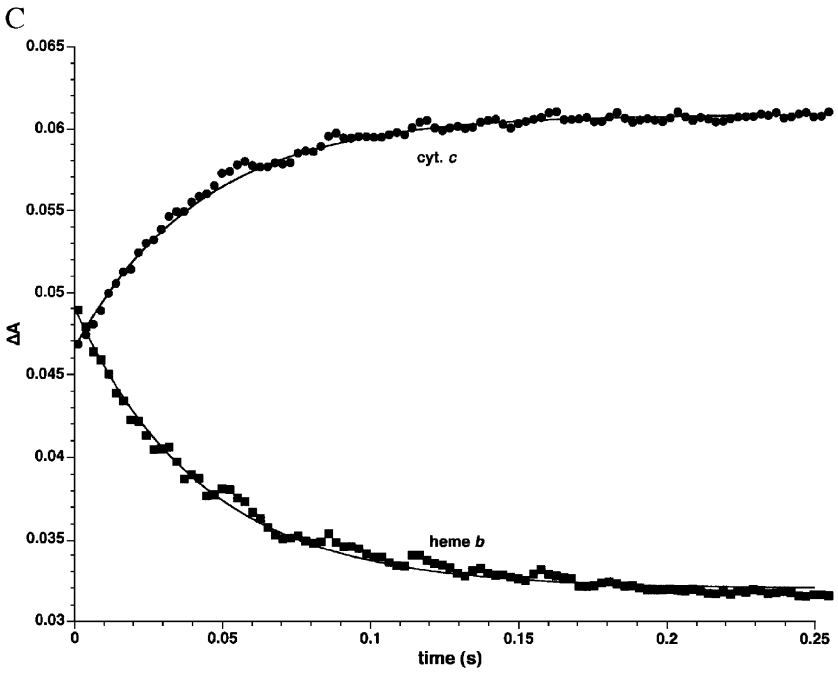


Fig. 1 (*continued*).

eliminated. Reduction of one-electron acceptors of this type via the heme group supports a chain model for inter-domain electron transfer in CBOR.

2. Materials and methods

2.1. Materials

Cytochrome *c* (horse heart, Type VI, prepared without the use of trichloroacetic acid) and ascorbic acid (sodium salt) were Sigma products. Buffer salts and other reagents were of AnalaR™ or similar quality.

2.2. Enzyme preparation

CBOR was prepared from the supernatant growth medium of cultures of *P. chrysosporium* (Commonwealth Mycology Institute 174727) as described by Jones and Wilson [11]. Under these growth conditions the CBOR is the only heme-containing secreted protein. The peroxidases (lignin peroxidase and manganese peroxidase) are produced after longer growth times under slightly different conditions [12]. CBOR heme domain was prepared as described previously by Hallberg et al. [10].

2.3. Spectrophotometry

2.3.1. Steady state spectrophotometry

Steady state spectrophotometry was carried out with a Hewlett-Packard diode array spectrophotometer HP8543 linked to a computer. Data analysis was carried out either with the resident HPChem software or by exporting the data as Microsoft Excel files.

Extinction coefficients determined or employed were:

ΔE_{mM} (563–571 nm), reduced *minus* oxidized, for heme $b = 24.0$

ΔE_{mM} (563 nm), reduced *minus* oxidized, for heme $b = 22.0$

ΔE_{mM} (550–540 nm), reduced *minus* oxidized, for cytochrome $c = 21.2$

ΔE_{mM} (431 nm), for the domain, reduced *minus* oxidized, for heme $b = 88$

ΔE_{mM} (550 nm), reduced *minus* oxidized, for cytochrome $c = 19.6$

The extinction coefficients at these wavelengths for the heme *b* group (cf. Ref. [13]) were essentially identical for intact enzyme and heme domain.

2.3.2. Stopped flow spectrophotometry

Stopped flow studies were carried out with an Applied Photophysics stopped flow spectrophotometer in either diode array or single wavelength mode. The diode array mode permits simultaneous monitoring of both heme and flavin while the repetitive single wavelength mode allows more accurate determination of absolute absorbance changes. 550 nm is the α-maximum of cytochrome *c* and also close to isosbesticity for oxidoreduction of heme *b*. 431 nm is isosbestic between ferric and ferrous cytochrome *c* in this apparatus, and also close to the Soret maximum in the reduced–oxidized difference spectrum of CBOR heme *b*. Output was displayed on the attached Acorn RISC computer

or transferred to a Macintosh computer; data analysis employed either the resident Pro-K™ software on the Acorn computer or Deltagraph™ 4.0 on the Macintosh. Rates for the fast electron transfer reactions were averages of at least three stopped flow kinetic traces.

3. Results

3.1. Reaction of cellobiose-reduced CBOR with ferricytochrome c

Fig. 1A shows the time course of cytochrome *c* reduction by CBOR with cellobiose as reductant for five different levels of ferricytochrome *c* at a constant total cytochrome *c* concentration. As expected from the reported rapid reduction rates of cytochrome *c* by the enzyme [5] and the slow electron transfer from flavin to heme [14], the time courses are almost zero order until very high levels of cytochrome *c* reduction are attained. Fig. 1B plots the measured rates during a continuous overall reaction at a constant total cytochrome *c* level as a function of [ferricytochrome *c*]. The rate is [ferricytochrome *c*] independent until the latter decreases below 1 μM. The fitted plot in Fig. 1B gives a K_m of 0.6 μM and a maximal rate of 0.63 μM cellobiose/min, or k_{cat} (electrons/CBOR/second) = 2.4 s^{-1}. When the total cytochrome *c* concentrations is varied, the maximal turnover is sometimes found to be dependent upon the total (ferri + ferro) cytochrome *c* concentration (not shown); this effect, a probable consequence of non-specific polycation activation, can give rise to misleadingly higher apparent Michaelis constants.

3.2. Reaction of ascorbate-reduced CBOR with ferricytochrome c

Holoenzyme CBOR was partially reduced with ascorbate; static spectrophotometry confirmed that only the heme group is reduced under these conditions. Aliquots were then mixed with oxidized cytochrome *c* in the stopped flow spectrophotometer, both at pH 6, the condition used for a number of steady state experiments (Fig. 1A and B), and at pH 4, as used for the heme domain experiments (below). A typical result is shown in Fig. 1C. The initial rapid reduction of cytochrome *c* is synchronous with the oxidation of CBOR heme *b*; thereafter, the two heme groups remain near equilibrium during a slow transition to near full reduction. A redox potential difference of 80–100 mV [15,16] ensures that at least 85% oxidation of heme *b* and a corresponding 85% reduction of cytochrome *c* occur at equilibrium. The rate constant of 23 s^{-1} at pH 6 is much higher than the overall maximal rate of cellobiose oxidation, limited by the rate of electron transfer from flavin to heme [14,16]. The calculated second-order constant of 8×10^6 M^{-1} s^{-1} is closely similar to that obtained from the overall reaction kinetics (Table 1). One equivalent of heme *b* is oxidized in the initial fast phase for each equivalent of cytochrome *c* reduced. In contrast to the cellobiose reaction [5], no reduction or reoxidation of the flavin was observed with ascorbate, either before or after cytochrome *c* addition (not shown).

3.3. Reaction of CBOR heme domain with ferricytochrome c

Fig. 2 illustrates the corresponding reactions with ascorbate-reduced purified heme domain. Rapid reduction of cytochrome *c* and reoxidation of heme *b* occurs as with intact enzyme. The calculated first-order rate constant of 100 s^{-1} (pH 4.0) represents a bimolecular rate constant of 4×10^6 M^{-1} s^{-1} for interaction of domain and cytochrome *c* (Table 1). A split time course trace (Fig. 2A) shows the complete process. At equimolar heme concentrations, all the initial (rapid) time courses fit second-order equations (not shown) while at the higher cytochrome *c* levels the time courses are exponential. A rapid initial reaction (oxidation of heme *b* and reduction of cytochrome *c*) is followed by a subsequent near-equilibrium and a final rereduction of both

Table 1
Rate constants for cyt. *c* reduction by CBOR and its isolated heme domain

Protein	Method	Reductant	Medium	pH	k_{max}obs (s^{-1})	k_{for} (M^{-1} s^{-1})[a]	K_{eq}obs[b]	k_{rev} (M^{-1} s^{-1})[c]
Intact CBOR	steady state	cellobiose	0.05 M phosphate, 22 °C	5.5–6.0	≈ 2.0[d]	4×10^6	ND[e]	ND[e]
Intact CBOR	stopped flow	ascorbate	0.1 M phosphate, 22 °C	6.0	>23[f]	8×10^6	ND[e]	ND[e]
Intact CBOR	stopped flow	ascorbate	0.17 M acetate, 0.17 M NaCl, 22 °C	4.0	>30[f]	2.3×10^6	≥40	ND
Heme domain	stopped flow	ascorbate	0.17 M acetate, 0.17 M NaCl, 22 °C	4.0	>100[f]	4×10^6	≥40	$\leq 2 \times 10^5$

[a] k_{for} (M^{-1} s^{-1}) is the calculated second-order rate constant for cyt. *c* interaction with reduced heme *b*.
[b] K_{eq}obs (dimensionless) is the apparent equilibrium constant for CDH heme and cyt. *c*, where $K(eq) = [c^{2+}] \times [b^{3+}]/[c^{3+}] \times [b^{2+}]$.
[c] k_{rev} is the reverse reaction rate constant obtained by mixing reduced cyt. *c* and heme domain or intact enzyme.
[d] k_{max}obs (steady state) is the maximal observed first-order electron transfer rate from enzyme to cyt. *c*; the rate saturation is attributed to the rate-limiting intramolecular electron transfer from flavin to heme *b*.
[e] At pH values ≥5.5, the potential difference between heme *b* and cytochrome *c* is ≥120 mV, the equilibrium constant ≥100, and the reverse reaction therefore very slow (cf. Ref. [16]).
[f] k_{max}obs (stopped flow) is the maximal observed first-order electron transfer rate between heme *b* and cyt. *c*; no rate saturation indicative of a functional binding and intracomplex electron transfer was observed.

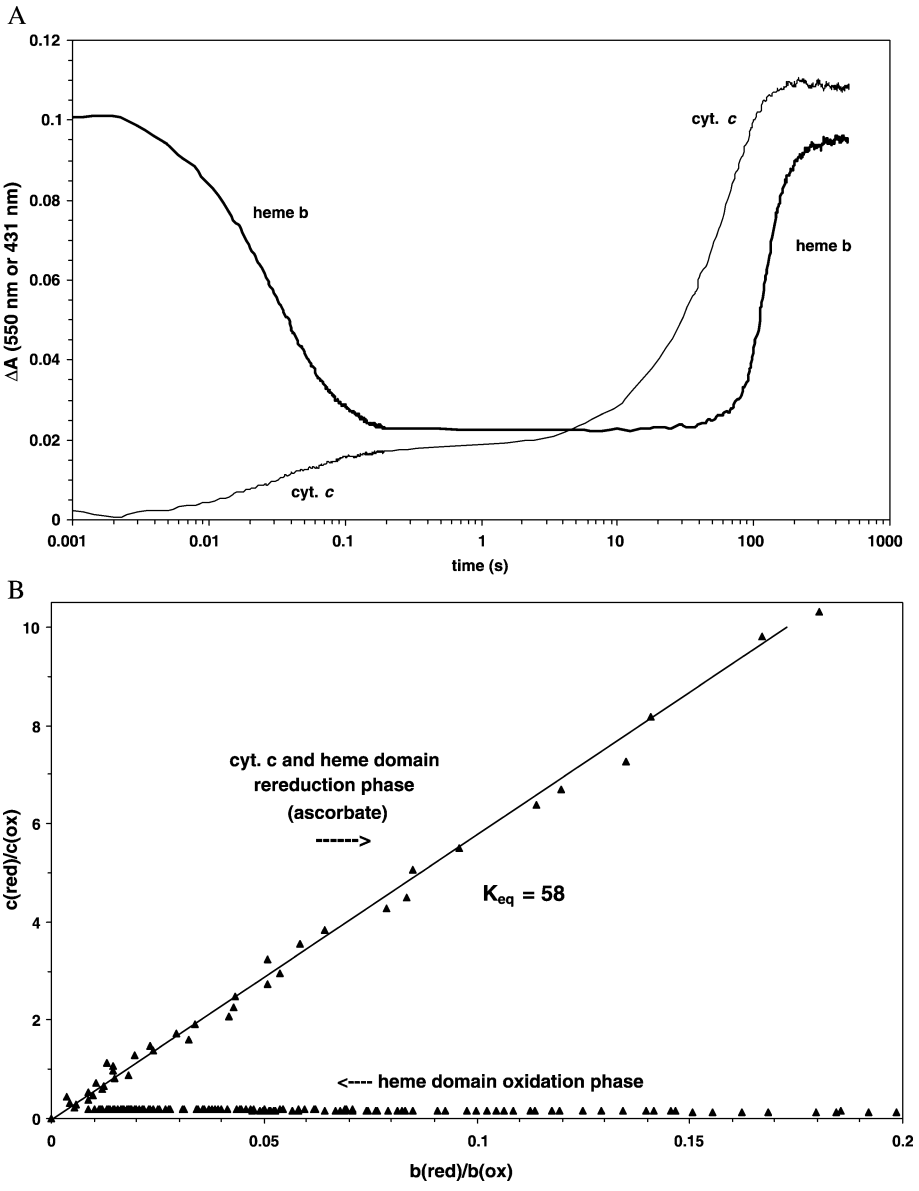


Fig. 2. The forward reaction:oxidation of ascorbate-reduced cellobiose oxidoreductase heme domain by ferric cytochrome *c*. Reduction of cyt. *c* in pH 4.0 0.17 M acetate buffer plus 0.17 M NaCl at 22 °C. Final concentrations: 1.55 μM heme *b*; 40 mM Na ascorbate; 6.7 μM ferric horse heart cytochrome *c*. Applied Photophysics stopped flow data. (A) Logarithmic time course: fast initial and final rereduction processes. Heavy gray line: heme *b* reduction monitored at 431-nm narrow black line; cytochrome *c* reduction monitored at 550 nm. (B) Calculated approach to equilibrium between the two heme proteins. Data from the experiment in (A) replotted as $[b^{2+}]/[b^{3+}]$ vs. $[c^{2+}]/[c^{3+}]$.

cytochrome *c* and heme *b* by excess ascorbate. In the last phase, cytochrome *c*, with the more positive redox potential, is reduced first; this is followed by the reduction of heme *b*. Fig. 2B shows apparent values of $[c^{2+}] \times [b^{3+}]/[c^{3+}] \times [b^{2+}]$ during the time course. After the initial fast phase the system approaches an equilibrium in which the equilibrium constant, $K_{eq}=[c^{2+}] \times [b^{3+}]/[c^{3+}] \times [b^{2+}]$, remains at a stable value between 55 and 60 despite a large variation in the absolute reduction levels of the two hemes. This corresponds to a difference in redox potentials of 100–105 mV. If E_0' (*c*)=+260 mV, E_0' (heme *b*) is close to +160 mV (cf. Ref. [16]).

3.4. Reaction of CBOR heme domain with ferrocytochrome c

Fig. 3 shows the reverse reaction when ascorbate-reduced ferrocytochrome *c* is mixed with oxidized CBOR domain. Heme *b* reduction proceeds rapidly (see inset) until the two systems are near equilibrium. A slow reduction by excess ascorbate then ensues, with the two hemes remaining near equilibrium, but with cytochrome *c* rereduced prior to rereduction of the domain. Between 30% and 40% of the heme domain present was reduced by added ferrocytochrome *c*, depending upon the latter's initial concentration. The experimental K_{eq} (pH 4) lay between 40 and 60 in

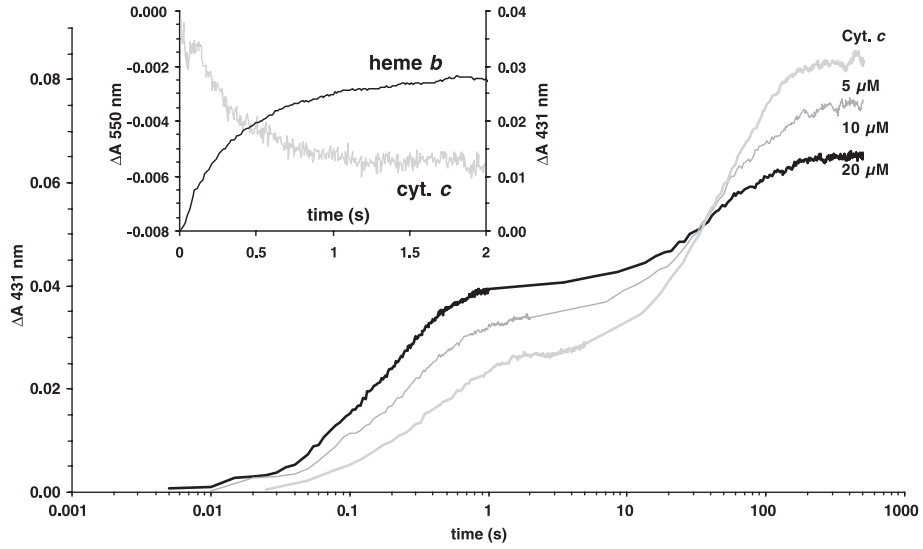


Fig. 3. The reverse reaction:oxidation of ascorbate-reduced cytochrome *c* by the cellobiose oxidoreductase heme domain. Ascorbate-reduced ferrocytochrome *c* was mixed with oxidized CBOR domain; reduction of the latter at 431 nm proceeds until the two systems are at equilibrium; 1.55 μM heme *b* (CBOR domain); cytochrome *c* reduced by 40 mM ascorbate; the two remain in equilibrium to near full reduction; pH 4.0; 22 °C. Note fast initial and final reduction of domain heme *b*. Logarithmic time scale as in Fig. 2A. Other conditions as in Fig. 2. Continuous heavy dark line: 20 μM ferrocyt. *c*. Continuous narrow dark line: 10 μM ferrocyt. *c*. Continuous heavy gray line: 5 μM ferrocyt. *c*. Inset: initial time course of reduction of 1.55 μM heme *b* by 5 μM ferrocyt. *c*. Linear time scale. Continuous dark line: heme *b* at 431 nm. Continuous gray line: cyt. *c* at 550 nm.

favour of cytochrome *c* reduction. This value is consistent with that obtained in the forward direction (Fig. 2B). While the final reduction of cytochrome *c* is essentially complete, that of the heme domain remains only partial if the reaction is carried out aerobically. This reflects rapid reoxidation of reduced heme *b* that occurs, due to reactive oxygen species (O_2^- and H_2O_2) in solution [11,17].

Fig. 4 summarises the kinetic data for forward and reverse reactions obtained with the isolated domain and the holoenzyme in the same medium. The averaged rate constants for cytochrome *c* reduction and for domain heme *b* oxidation are plotted as a function of cytochrome *c* concentration. Straight lines, with no sign of saturation ($k_{max} \geq 100\ s^{-1}$) at the highest cytochrome *c* concentrations, give a bimolecular rate

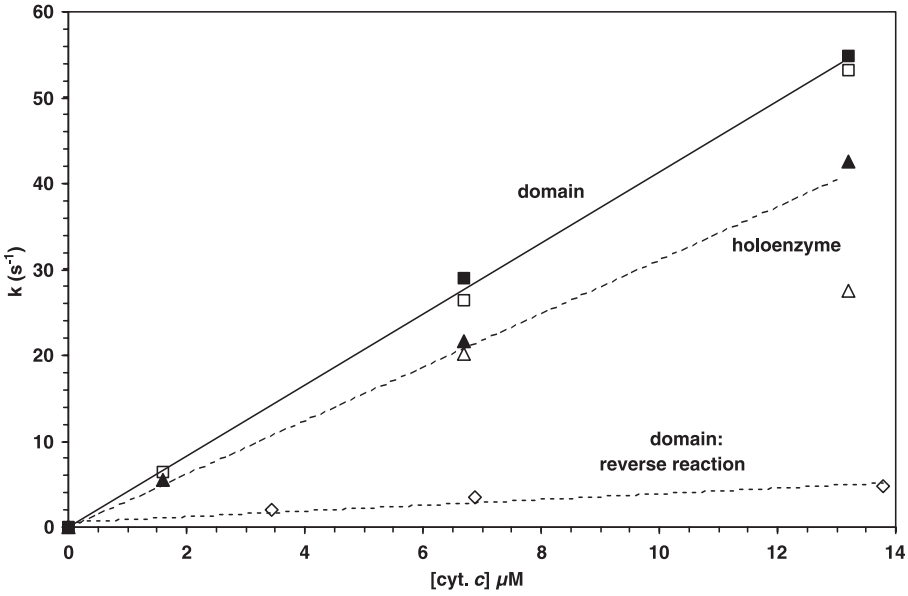


Fig. 4. Effect of cytochrome *c* concentration on the reaction with cellobiose oxidoreductase heme domain in ascorbate-reduced and oxidized states. Computed rate constants for forward and reverse reactions from experiments such as those in Figs. 2 and 3, at pH 4.0 and 22 °C, are plotted as a function of cytochrome *c* concentration. Other conditions as in Figs. 2 and 3. (■—■) forward reaction:oxidation of domain heme *b* (431 nm); (□—□) forward reaction:reduction of cyt. *c* by domain (550 nm); (▲– – –▲) forward reaction: oxidation of holoenzyme heme *b* (431 nm); (△– – –△) forward reaction:reduction of cyt. *c* by holoenzyme (550 nm); (◇- - - -◇) reverse reaction:oxidation of domain heme *b* (431 nm).

constant of 4×10^6 M^{-1} s^{-1} for the domain and 2.3×10^6 M^{-1} s^{-1} for intact CBOR. The rates of ferrocytochrome *c* oxidation by oxidized domain (cf. Fig. 3) at different cytochrome *c* concentrations give a rate constant for the reverse reaction of 1.5×10^5 M^{-1} s^{-1}, consistent with the estimated equilibrium constant (Fig. 3).

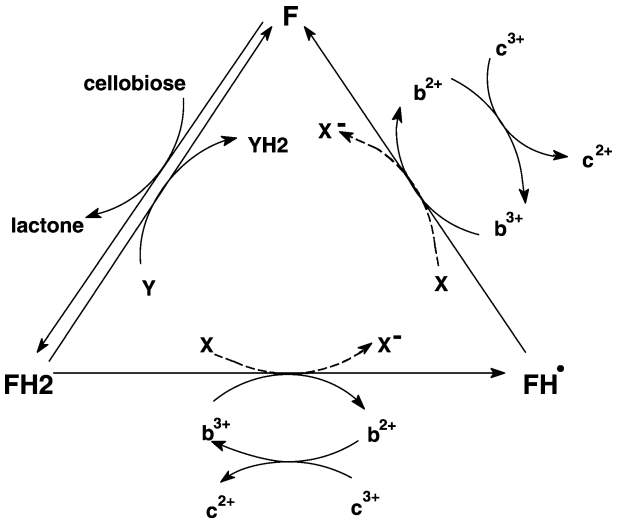
4. Discussion

The results show conclusively that the anionic heme *b* domain of CBOR can rapidly transfer electrons to cationic cytochrome *c*. Under the ionic conditions employed this occurs by a collision process, presumably involving the heme edges of the two proteins. The stability of the resulting complex is low (CBOR never meets with cytochrome *c* in its natural life) and so first-order behaviour is seen up to relatively high levels of cytochrome *c*. The final redox equilibrium between the two heme proteins at pH 4 is consistent with a potential difference of approximately 100 mV (CBOR heme less positive), in accordance with previous measurements and estimates [15,16]. Rates of cytochrome *c* reduction with cellobiose as reductant under catalytic (turnover) conditions have also been determined (Fig. 1 and unpublished data). These show that the rate of cytochrome *c* reduction is typically much faster than any of the other steps in the enzymatic turnover.

Cytochrome *c* in these experiments acts as a convenient analogue of possible physiological acceptors such as ferric salts, especially of the cationic type [18,19]. The electron donor ascorbate short-circuits the flavin group in the intact enzyme and is essential for reduction of the heme in the isolated domain, which does not react with cellobiose. The domain then acts as an ascorbate-cytochrome *c* reductase, as the rate of reduction of CBOR heme by ascorbate is greater than the reduction of cytochrome *c* itself (approximately 1.0 M^{-1} s^{-1} compared to 0.3 M^{-1} s^{-1} at pH 4; unpublished results).

Reduction of several acceptor types by the holoenzyme [5,6,18] may thus proceed as in Scheme 1. Cationic ferric complexes such as cytochrome *c* always accept electrons from the heme domain. Removal of electrons from the flavin domain can be carried out (i) intramolecularly by heme *b*; (ii) by two-electron acceptors (Y); or (iii) by neutral and possibly by anionic one-electron acceptors (X). Reduction of oxygen may be of either kind; a two-electron reduction (type Y) gives hydrogen peroxide [20] while a one-electron process (type X) gives superoxide [17]. The predicted flavin radical (Scheme 1) was reported by Cameron and Aust [8] and confirmed by Mason et al. [17]. Reduction pathways for other acceptors remain to be determined. The most important "ecological" acceptor has yet to be identified [1].

What therefore does the heme domain do? It generates ferrous iron species by reduction of the model acceptor cytochrome *c* and possibly in the reduction of functional iron salts such as ferric oxalate [4]. Ferrous complexes can then react with peroxides, the latter being produced either by CDH itself (cf. Scheme 1) or by other flavin oxidases, and thereby generate either hydroxyl radicals or ferryl iron, both Fenton-type oxidants that can assist in lignin degradation.



Scheme 1. The cellobiose oxidoreductase catalytic cycle. The oxidized flavin domain (F) can be reduced by cellobiose in a two-electron process to give reduced flavin (FH2). This can be reoxidized by a two-electron acceptor (Y), such as dioxygen or a quinone, to restore the oxidized species, or by either an external one-electron acceptor (X), or the associated heme domain (*b*), to give the semiquinone form of the flavin (FH•). Heme *b* can be reoxidized by added cationic acceptors such as cytochrome *c*.

Acknowledgements

We thank Dr. Gareth Jones of the University of Essex (UK) for advice concerning CBOR preparation, Dr. Martin Krusaa of the Pulp and Paper Institute at KTH (Stockholm, Sweden) for discussions of CBOR (CDH) functioning as a lignin degrading system, and Drs. Gunnar Johansson and Goran Pettersson of Uppsala University (Sweden) for discussion of the molecular biology of the system. MGM acknowledges the support of her work by a BBSRC (UK) postgraduate studentship.

References

[1] G. Henriksson, G. Johansson, G. Pettersson, J. Biotechnol. 78 (2000) 93–113.
[2] V. Massey, J. Biol. Chem. 269 (1994) 22459–22462.
[3] Q. Su, J. Klinman, Biochemistry 38 (1999) 8572–8581.
[4] M.T. Wilson, B.-L. Liu, Biochem. Soc. Trans. 22 (1994) 725–728.
[5] M.S. Rogers, G.D. Jones, G.M. Antonini, M.T. Wilson, M. Brunori, Biochem. J. 298 (1994) 329–334.
[6] G. Henriksson, G. Pettersson, G. Johansson, A. Ruiz, E. Uzcategui, Eur. J. Biochem. 196 (1991) 101–106.
[7] G. Henriksson, G. Johansson, G. Pettersson, Biochim. Biophys. Acta 1144 (2000) 184–190.
[8] M.D. Cameron, S.D. Aust, Biochemistry 39 (2000) 13595–13601.

[9] M. Samejima, K.E.L. Eriksson, Eur. J. Biochem. 207 (1992) 103–107.
[10] B.M. Hallberg, T. Bergfors, K. Bäckbro, G. Pettersson, G. Henriksson, C. Divne, Structure 8 (2000) 79–88.
[11] G.D. Jones, M.T. Wilson, Biochem. J. 256 (1988) 713–718.
[12] F.H. Perle, M.H. Gold, Appl. Environ. Microbiol. 57 (1991) 2240–2245.
[13] G. Henriksson, L. Zhang, J. Li, P. Ljungquist, T. Reitberger, G. Pettersson, G. Johansson, Biochim. Biophys. Acta 1480 (2000) 83–91.
[14] K. Igarashi, I. Momohara, T. Nishino, M. Samejima, Biochem. J. 365 (2002) 521–526.
[15] J.D. Wood, P.M. Wood, Biochim. Biophys. Acta 1119 (1992) 90–96.
[16] K. Igarishi, M.F.J.M. Verhagen, M. Samejima, M. Schulein, K.-E.L. Eriksson, T. Nishino, J. Biol. Chem. 274 (1999) 3338–3344.
[17] M.G. Mason, M.T. Wilson, A. Ball, P. Nicholls, FEBS Lett. 518 (2002) 29–32.
[18] M.T. Wilson, B.-L. Liu, Biochem. Soc. Trans. 22 (1994) 725–728.
[19] M.T. Wilson, N. Hogg, G.D. Jones, Biochem. J. 270 (1990) 265–267.
[20] A. Nutt, A. Salumets, G. Henriksson, V. Sild, G. Johansson, Biotechnol. Lett. 19 (1997) 379–383.