Reactivity and Redox Potential of Heme-Thiolate Proteins – Results from Enzymes and Enzyme Models

Wolf-D. Woggon

Abstract: The redox potential is a characteristic parameter of various intermediates of the catalytic cycle of heme-thiolate proteins (cytochromes P450, NO-synthase, chloroperoxidase) which significantly influences catalytic turnover. \(E_\text{red}\) values of these proteins are surprisingly positive compared to synthetic active site analogues that have an arylthiolate or an alkylthiolate coordinating to the iron. This report examines factors underlying this phenomenon and describes the design of enzyme mimics having redox potentials close to those of heme-thiolate proteins.

Keywords: Cytochrome P450 · Enzymes · Enzyme models · Iron porphyrins · Iron-sulfonate coordination · Iron-thiolate coordination · Redox potential

Introduction

Heme-thiolate proteins comprise the enzymes cytochrome P450 [1], NO-synthase [2], and chloroperoxidase (CPO). The two first proteins are extremely important to mammals due to their central role in the metabolism of drugs/synthesis of hormones and the production of \(\cdot\)NO as a signal messenger, respectively. In contrast CPO has attracted attention mainly because of its diverse reactivity profile [3] and its peculiar reaction mechanism [4–6].

Current knowledge regarding the various catalytic reactions of heme-thiolate proteins [7][8] rests to a large extent on studies of cytochrome P450 [9]. This cytosolic protein can be easily purified from Pseudomonas putida and has been overexpressed in other bacteria [10]. X-ray structures of various forms of P450 [11–16] have been obtained, providing touchstones for understanding the topology at the active site. The enzyme contains an iron(III) protoporphyrin(IX) complex 1 bound to the protein via its two propionate site chains and a thiolate ligand from Cys357 coordinating to the iron from the proximal site (Scheme 1). Since its early discovery, the significance of the thiolate ligand for the chemical reactivity of the system [17], and its influence on the enzyme’s UV and EPR spectral properties and electrochemistry have been a matter of debate and have initiated intense investigations [18].

The catalytic cycle of P450 [18] (Scheme 1), and hence that of heme-thiolate proteins more generally, has been established by taking into account the X-ray structures and Laue snapshots [19] of certain intermediates, numerous mechanistic studies on various P450s and chemical models thereof [18]. Accordingly, the sequence of events in the catalytic cycle of the heme-thiolate proteins is certain up to intermediate 2.

Subsequent steps, though lacking solid characterization, have been deduced from studies using site-specific mutagenesis, by comparing spectroscopic parameters of enzymes and model compounds, and by measuring isotope effects. The formation of a high-valent iron(IV) oxo porphyrin radical cation intermediate 3 by protonation of the peroxy complex 2 and subsequent O–O bond cleavage is in agreement with data from different sources. By analogy to model studies [18] this intermediate is believed to have electronic structure 3 rather than 4. For the past two decades, the mechanism of P450-catalyzed hydroxylations has been described as a two-step reaction of 3 with the substrate: hydrogen abstraction by the iron-oxo of 3 gives a substrate alkyl radical 6 that is immediately trapped by HO· from the iron, yielding the hydroxylated substrate 7 and the water-free form of the enzyme in its resting state 1 [18]. Recently, however, several aspects of the catalytic cycle have been questioned, including: i) the origin of the low-spin state of 5 [20], the resting state of P450 [21], ii) the electronic nature of compound 3/4 [18][21], and iii) the two-step oxygen-rebound mechanism [22][23]. In the present account we focus on the significance of the redox potential of P450 intermediates and corresponding synthetic active site analogues.

Results and Discussion

Three steps of the reaction sequence shown in Scheme 1 are obviously redox sensitive: i) reduction of iron(III) 9 \(\to\) iron(II) 10, ii) addition of an electron to the terminal oxygen bound to iron (11 \(\to\)
2), and the interaction of 3/4 with the substrate.

The redox potential of 3/4 is not known but, since it is a strong oxidant, it can abstract H+ from substrates (Scheme 1), as well as accept electrons from suitable substrates such as amines [18]. The transformation of 11 → 2 is required to initiate oxygen cleavage, and it is known in certain cases that the donation of an electron to 11 is rate limiting, leading to a small, not fully expressed kH/kO isotope effect or H+ removal [18], for example in the hydroxylation of camphor (Scheme 1).

The six-coordinate resting state of P450cam (5) is an important intermediate in the catalytic cycle. Due to the presence of the thiolate ligand its redox potential is very negative (Eo = -290 mV) [24], precluding reduction by NADPH via putidaredoxin [25][26]. Thus, 5 is essentially inert with respect to catalysis and is only activated upon removal of the coordinating water and subsequent binding of the substrate camphor 8 to yield the high-spin iron(III) complex 9 [26–28] (Eo = -170 mV) [23] which can be subsequently reduced to 10. This fine-tuning of the redox potential of 5 to -290 mV [24], one of the most elegant examples of gating biological electron transfer, is accomplished by two factors. First, the iron(III) adopts a low-spin state, and second, the thiolate ligand provided by Cys357 is hydrogen bonded to two amino acids from the protein [27]. The latter aspect, not immediately recognized in the first X-ray structures of P450cam, was predicted from the Eo values of the synthetic active site analogues 12 [28] and 13 [29] (Fig.). Both display rather negative redox potentials (-607 and -714 mV), indicating a clear correlation between Eo and the effective charge at S-.

Several possibilities exist for generating catalytic enzyme models having Eo values close to P450cam (Eo = -290 mV [24]) or CPO (Eo = -140 mV [30]). The synthetically most convenient approach involves the preparation of electron-deficient porphyrins like 14 and 15 (Fig.). Nitro-substituted iron porphyrins, derived from parent compound 15, provide access to enzyme models with Eo values
between -500 and -200 mV depending on the degree of nitro substitution. However, these complexes are rather difficult to prepare [31] by direct nitration of the porphyrin. In contrast, model compound 14, containing two pentafluorophenyl substituents as electron-withdrawing groups in the meso-position of the porphyrin plane, is easily accessible and exhibits a redox potential (Eo = -134 mV) comparable to that of CPO [32].

From these model studies we can conclude that Eo is largely dependent on the electron-donating character of the proximal S-ligand (e.g. 15, with a para-NO2 substituted thiophenolate ligand) and the structure of the porphyrin and can be further modulated by substituents protecting the distal site of the porphyrin. It is interesting to note that the ΔEo between 12 and 16 [33] is about 100 mV suggesting that Eo depends on the polarity of the distal site. Remarkable anodic shifts are observed only if the electron density at S- is reduced, as in 15, or electron donation from the porphyrin ligand is reduced, as in 14 and 15. If these arguments are also valid for P450cym, then the main contribution to the ΔEo of 400 mV between the enzyme's resting state and the model compound can be attributed to H-bonding to the thiolate of cysteine 357. Smaller effects may result from the protein environment of the distal substrate pocket.

The catalytically important 120 mV anodic shift 7 → 9, however, is associated with the change from a low-spin to a high-spin state. Though analogues have been prepared (Eo = -350 mV) that mimic the unique S-cys'-H-N bonding of P450cym, these models are unsuitable for catalytic reactions [34][35].

A third possibility for varying the redox potential of heme-thiolate proteins involves a very convenient modification of the thiolate ligand, i.e. oxidation to the sulfonate SO3- (Scheme 2). In agreement with our expectations, by distributing the single negative charge of S- (17) over a much larger space volume in the SO3- group, a very significant anodic shift of 285 mV to Eo = -175 mV (see 18) can be achieved which, within experimental error, corresponds exactly to the value of 9,

Fig. Redox potentials (Eo) of various synthetic enzyme models of heme-thiolate proteins. Eo obtained from cyclic voltammograms
the high-spin E S complex of cytochrome P450cam.

Interestingly these unusual modifications of the heme-thiolate model system produced good catalysts for epoxidations both in homogenous (17-PhIO, 18-PhIO) and heterogenous fashion (19-PhIO) [36].

Acknowledgements

I thank the PhD students who have contributed significantly to the results described in this article, in particular Dr. Beat Stübbi, Dr. Sandro Ghirlanda, Dr. H.-A. Wagenknecht, Dr. H. Aissaoui, Dr. Olivia Forrer, and Tycho R. Lyfels. This research was supported by the Swiss National Science Foundation, and F. Hoffmann-La Roche AG, Basel.

Received: February 22, 2001

[1] 'Cytochrome P450. Structure, Mechanism, and Biochemistry', Ed. P. Ortiz de Montellano, 2nd ed., Plenum, New York, 1995.

[2] 'Cytochrome P450. Structure, Mechanism, and Biochemistry', Ed. P. Ortiz de Montellano, Plenum, New York, 1986.

[3] M.C.R. Franssen, Biocatalysis 1994, 1087-1110, and references therein.

[4] H.-A. Wagenknecht, W.-D. Woggon, Angew. Chem. Int. Ed. Engl. 1997, 36, 390-392.

[5] H.-A. Wagenknecht, W.-D. Woggon, Chem. Biol. 1997, 4, 367-372.

[6] H.-A. Wagenknecht, C. Claude, W.-D. Woggon, Helv. Chim. Acta 1998, 81, 1506-1520.

[7] J.H. Dawson, M. Sono, Chem. Rev. 1987, 87, 1255-1276, and references therein.

[8] M. Sono, M.P. Roach, E.D. Coulter, J.H. Dawson, Chem. Rev. 1996, 96, 2841-2887, and references therein.

[9] 'Cytochrome P450. Structure, Mechanism, and Biochemistry', Ed. P. Ortiz de Montellano, 2nd ed., Plenum, New York, 1995.

[10] L.-L. Wong, A.C.G. Westlake, D.P. Nickerson, Structure and Bonding 1997, 88, 175-205, and references therein.

[11] T.L. Poulos, B.C. Finzel, I.C. Gunsalus, G.C. Wagner, J. Kraut, J. Biol. Chem. 1985, 260, 6122-6130.

[12] T.L. Poulos, B.C. Finzel, A.I. Howard, Biochemistry 1986, 25, 5314-5322.

[13] T.L. Poulos, B.C. Finzel, A.I. Howard, J. Mol. Biol. 1987, 195, 687-700.

[14] R. Raag, T.L. Poulos, Biochemistry 1989, 28, 917-922.

[15] R. Raag, T.L. Poulos, Biochemistry 1989, 28, 7586-7592.

[16] R. Raag, T.L. Poulos, Biochemistry 1991, 30, 2674-2684.

[17] J.H. Dawson, Science 1988, 240, 433-439, and references therein.

[18] W.-D. Woggon, Top. Curr. Chem. 1996, 184, 39-96, and references therein.

[19] I. Schlichting, J. Berendzen, K. Chu, A.M. Stock, S.A. Maves, D.E. Benson, R.M. Sweet, D. Ringe, G.A. Petsko, S.G. Sligar, Science 2000, 287, 1615-1622.

[20] H. Aissaoui, R. Bachmann, A. Schweiger, W.-D. Woggon, Angew. Chem. Int. Ed. 1998, 37, 2998-3002.

[21] M.T. Green, J. Am. Chem. Soc. 1999, 121, 7939-7940.

[22] M. Newcomb, P.H. Toy, Acc. Chem. Res. 2000, 33, 449-455.

[23] S. Shaik, M. Filatov, D. Schröder, H. Schwarz, Chem. Eur. J. 1998, 4, 193-199.

[24] S.G. Sligar, I.C. Gunsalus, Proc. Natl. Acad. Sci. USA 1976, 73, 1078-1082.

[25] M.J. Hintz, J.A. Peterson, J. Biol. Chem. 1981, 256, 6721-6728.

[26] M.J. Hintz, D.M. Mock, L.L. Peterson, K. Tuttle, J.A. Peterson, J. Biol. Chem. 1982, 257, 4324-4332.

[27] T.L. Poulos, J. Biol. Inorg. Chemistry 1996, 1, 356-359.

[28] B. Stübbi, H. Fretz, U. Piantini, W.-D. Woggon, Helv. Chim. Acta 1997, 70, 1173-1193.

[29] S.L. Ghirlanda, Dissertation, Universität Zürich, 1994.

[30] R. Makino, R. Chiang, L.P. Hager, Biochemistry 1976, 15, 4748-4754.

[31] O. Forrer, Dissertation, Universität Basel, 1998.

[32] H.-A. Wagenknecht, C. Claude, W.-D. Woggon, Helv. Chim. Acta 1998, 81, 1506-1520.

[33] H. Aissaoui, S. Ghirlanda, C. Gnür, W.-D. Woggon, J. Mol. Catal. 1996, A, 113, 393-402.

[34] N. Ueyama, N. Nishikawa, Y. Yamada, T.-s. Okamura, A. Nakamura, J. Am. Chem. Soc. 1996, 118, 12825-12827.

[35] T. Uno, Y. Kousumi, K. Yoshizawa-Kunagaya, K. Nakajima, N. Ueyama, T.-A. Okamura, A. Nakamura, J. Am. Chem. Soc. 1998, 120, 12264-12273.

[36] T. R. Lyfels, W.-D. Woggon, in preparation.