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Journal of Inorganic Biochemistry 99 (2005) 2292–2298
JOURNAL OF Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

The mechanism of Compound I formation revisited

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Received 1 April 2005; received in revised form 10 July 2005; accepted 19 August 2005
Available online 4 October 2005

Abstract

The most recently proposed mechanisms for the formation of the Compound I intermediates of the peroxidases and catalases have been based on the crystallographic elucidation of the enzyme structures. It has been assumed that these mechanisms are compatible with an earlier proposal of the formation of a reversible enzyme–substrate intermediate called Compound 0, which was based on data that pre-dated the availability of the enzyme structures. However, it is argued here that this is not the case and some modifications of the existing mechanism are proposed which reconcile the structural, kinetic and energetic data for the reactions. This paper focuses attention on horseradish peroxidase isoenzyme C and particularly on the acid–base properties of the imidazole side chain of distal histidine 42. This imidazole group has an exceptionally low pK_a value in the resting enzyme, which is higher in Compound I and higher still in Compound II. The pK_a value must also be greatly increased following Compound 0 formation so that the imidazole can become an effective proton acceptor. An explanation is offered in a dielectric insertion (DI) model, in which the peroxide substrate, or fragments thereof, screens the influence of the positively charged heme iron on the pK_a value of the imidazole group. It is proposed that Compound 0 is converted to a second intermediate, Compound 0*, by intramolecular proton transfer along a pre-existing hydrogen bond, a process which reduces the energy requirements of charge separation in the deprotonation of hydrogen peroxide.

Keywords: Peroxidases; Catalases; Compound I; Proton transfer; Electron density shifts

1. Introduction

Studies of the nature, formation and reactivities of the key Compound I intermediates of the heme peroxidases and catalases have a long (almost 70 years) and tortuous history, which can be traced, in the main, to the fact that the curious chemistry of these remarkable species has sequentially confounded a variety of scientific preconceptions [1]. Thus, when peroxidase Compound I was first observed it was, almost inevitably, thought to be a reversible enzyme–substrate complex (Complex I) in accord with general ideas about enzyme mechanisms. When it turned out that the formation of the intermediate involves an irreversible (almost always) 2-electron equivalent oxidation of the enzyme (so that the designation Compound I became appropriate), some confusion reigned in the literature, particularly because it emerged that the location of the oxidising equivalents may be different in different enzymes. A common feature proved to be the presence of an Fe(IV) oxy-cation, ferryl, formed by oxidation of the Fe(III) ferriheme centre in the native enzymes. These were quite remarkable findings; oxidation states of iron other than Fe(II) or Fe(III) were thought to be so rare that they were either not mentioned, or received only brief comment in standard works on inorganic chemistry. The location(s) of the second oxidising equivalent (as a free radical site in most cases) provoked heroic endeavours which have substantially (but perhaps not always unequivocally) resolved the problem.

Almost 30 years ago, the present writers addressed the question of the mechanism of Compound I formation [2].

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doi:10.1016/j.jinorgbio.2005.08.009

This might appear to have been an unnecessary study since the reactions were often written (uninformatively) in the style,

$$\text{Native enzyme} + H_2O_2 \rightarrow \text{Compound I} \quad (1)$$

and it was implicitly assumed that the process is an elementary bimolecular reaction. Our discussion assessed the existing kinetic, energetic and thermodynamic data in the context of two basic principles of kinetic analysis: (1) the rate constant for an elementary bimolecular reaction cannot exceed diffusion control; (2) the activation energy for an elementary bimolecular reaction in solution must be equal to or greater than that for diffusion control. It could securely be concluded: (a) that the formation of Compound I species do not occur as elementary bimolecular reactions; (b) that they involve reaction of molecular hydrogen peroxide with enzyme; and (c) that a minimal acceptable mechanism involves the formation of a reversible enzyme–substrate complex. A precursor complex (termed Compound 0) was subsequently characterised [3,4] at sub-zero temperatures using cryosolvents of the type pioneered by Douzou [5] although its structure has remained controversial.

Our 1977 discussion preceded the elucidation of the first peroxidase and catalase crystal structures and the unravelling of many subtle facets of the structures by diverse spectroscopic techniques. This wealth of information might suggest the present article to be redundant, but we argue below that some important aspects of the mechanism of Compound I formation remain unclear and merit further examination.

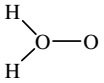
2. Discussion

2.1. Acid–base catalysis and the Poulos–Kraut mechanism

Characterisation of the nature of the redox reaction in Compound I formation as

$$\underset{\text{Native enzyme}}{\text{Protein–Porphyrin-Fe(III)}} + H_2O_2 \rightarrow \underset{\text{Compound I}}{\text{Protein–Porphyrin}(+^{\bullet})\text{-Fe(IV)=O}} + H_2O \quad (2)$$

where the ferryl centre (Fe(IV)=O) is present in all oxidized peroxidase/catalase species of this type and the porphyrin π-cation radical is present in many (e.g., horseradish peroxidase isoenzyme C, HRP C) but not all (e.g., yeast cytochrome *c* peroxidase, C*c*P) led to speculation about the roles of the proteins in the mediation of the reaction in the active site before either the primary or tertiary structures of the enzymes were known. The nature of the reaction strongly suggests that, during the process, hydrogen peroxide is converted from its usual skew chain form to a structure approaching the trigonal form,



(3)

Since, in this structure, the O–O bond order is zero its formation could scarcely arise other than at or near the reaction transition state. (Historically, the trigonal structure was, at first, held to be most likely for hydrogen peroxide, since it could account most readily for much of the reaction chemistry [6].) Nonetheless, the conversion essentially to trigonal hydrogen peroxide has the form of a prototropic isomerisation, so that general acid–base catalysis, perhaps of the ternary "push–pull" type championed by Swain [7], might be mediated by appropriate amino acid side chains in the active site [8].

The subsequent elucidation of the enzyme tertiary structures (firstly of yeast C*c*P) [9], and later mutant enzyme studies [10] appeared to some extent to support this view but also to offer an important simplification. Poulos and Kraut [11,12] advanced a mechanistic hypothesis in which the imidazole group of a distal histidine residue accepted the proton (H2) from the leading oxygen atom (O2) of a hydrogen peroxide molecule entering the active site (Fig. 1). Subsequently, the (H2) proton is transferred to the (O1) oxygen (in a trigonal transition state) thereby resulting in cleavage of the O—O bond and the formation of Compound I. The mutant enzyme studies [10] underscored the crucial role of the distal His in the reaction. Thus, the prototropic isomerisation of hydrogen peroxide envisaged in this mechanism invokes His-imidazole in base catalysis and (perhaps less essentially) in acid catalysis.

Analysis of the kinetics of Compound I formation as a function of pH [13] implies that the distal His-imidazoles in these enzymes are unusually acidic (pK_a (ImH^+) ~ 2.5 in HRP C and <4 in yeast C*c*P) and that only uncharged

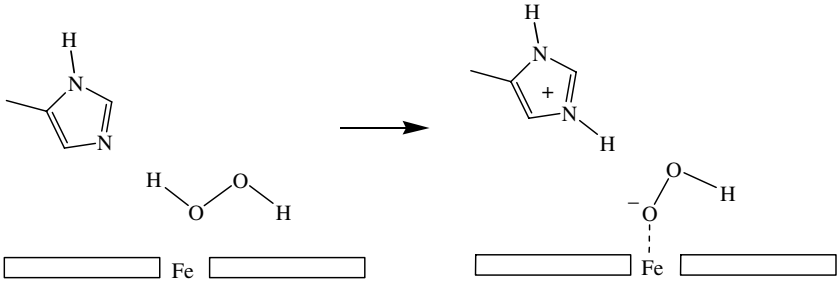


Fig. 1. First step in Compound I formation according to Poulos and Kraut [11,12]. Proton transfer occurs from hydrogen peroxide to the distal imidazole group and an iron-oxygen bond is formed.