How do enzymes work? Effect of electron circuits on transition state acid dissociation constants

Received: 23 January 2001 / Accepted: 17 April 2001 / Published online: 22 June 2001
© SBIC 2001

Abstract The effect of electron flow through a complete circuit on transition state acid dissociation constants is used to explain the remarkable catalysis observed in a redox reaction, the formation of compound I from native peroxidase. The explanation for the huge shift in the dissociation constant of a distal histidine residue, in going from the resting enzyme to the transition state, is a complete electron circuit through many amino acid residues and hydrogen bonds which prevents the development of localized charge. The key feature is electron flow through the circuit at the instant that proton transfer is occurring in the opposite direction. Electron flow occurs in one direction for attainment of the transition state and in the opposite direction for product formation.

Keywords Enzyme catalysis · Acid-base catalysis · Enzyme electron flow circuits · Transition state · Heme peroxidases

Introduction

Enzymes are the catalysts of life. In this article, an important way in which enzymes can be such efficient catalysts is described. Catalysis can be achieved by shifting the values of acid dissociation constants during the course of a reaction. When a cycle is completed, the values of all acid dissociation constants within the enzyme must return to their initial values. However, during the course of the reaction, dramatic shifts can occur, and the greater the shift, the greater the extent of catalysis.

During the course of an enzyme cycle, intermediate species may be formed and then disappear, as illustrated for the peroxidases. The peroxidases utilize hydrogen peroxide to oxidize single-electron or hydrogen atom donors (AH) according to the following mechanism:

\[ \text{Peroxidase} + \text{H}_2\text{O}_2 \rightarrow \text{Compound I} + \text{H}_2\text{O} \] (1)

\[ \text{Compound I} + \text{AH} \rightarrow \text{Compound II} + A^* \] (2)

\[ \text{Compound II} + \text{AH} \rightarrow \text{Peroxidase} + A^* + \text{H}_2\text{O} \] (3)

Several different types of chemistry govern the fate of the free radicals A*, depending on their structure [1]. A recent paper offers an explanation for the remarkably different mechanisms of peroxidases and the closely structurally related catalases [2]. The catalatic mechanism is:

\[ \text{Catalase} + \text{H}_2\text{O}_2 \rightarrow \text{Compound I} + \text{H}_2\text{O} \] (4)

\[ \text{Compound I} + \text{H}_2\text{O}_2 \rightarrow \text{Catalase} + \text{H}_2\text{O} + \text{O}_2 \] (5)

Both peroxidases and catalases have five-coordinate iron with no water in the sixth coordination position, despite having some water located in the distal pocket. Hydrogen peroxide, an ω-nucleophile [3], readily diffuses into the sixth coordination position of both native catalases and peroxidases to form compound I, whereas the structurally similar but less nucleophilic water molecules do not compete for the iron site. In both the peroxidases and catalases, water is released upon compound I formation. It is the speed with which this newly formed water molecule diffuses out of the active site that is claimed to determine the mechanistic differences. In the peroxidases, the water is retained so that reaction with a second hydrogen peroxide molecule is blocked. In the catalases the water diffuses away so that reaction with a second hydrogen peroxide occurs readily.

The subsequent discussion is restricted to one elementary reaction: the first step in compound I forma-
Peroxidase is used as the example, although the proposed features should also be relevant to compound I formation of catalases. A single acid-base group on the enzyme, the imidazole side chain of a distal histidine residue, is the key to catalysis. Catalysis can be attributed to the shift of pKₐ value of the histidine in going from the native enzyme to its transition state. The key question, for which I offer an explanation, is how the shift in pKₐ value is attained.

**Compound I formation in peroxidases**

Key parts of the active site structure of a peroxidase are illustrated schematically in Fig. 1 [4]. The heme in the active site consists of a porphyrin ring with an iron(III) in the center. The porphyrin is represented by the two bars. The iron is bonded to the imidazole ring of a histidine residue located on the proximal side below the heme. Another histidine residue is located above the heme on the distal side. Its imidazole ring is not bonded to the iron(III). The distal imidazole ring is the key base responsible for catalysis of compound I formation. In the overall reaction, hydrogen peroxide binds in the active site, donates an oxygen atom to the iron(III), and water is released. The iron attains a formal oxidation state of +5, but donation of an electron from the porphyrin ring to iron leads to formation of a ferryl group (Fe⁴⁺ = O) and a porphyrin π-cation radical, key features of the compound I structure [5, 6].

The pKₐ value of the distal imidazole in the native enzyme is approximately 2.5. Above pH 2.5, the rate of compound I formation is pH independent [7]. The key to the catalysis is the dramatic shift in value of this pKₐ as the transition state is attained, and its return to a low value as the reaction is completed. How can this pKₐ value be shifted and by how much?

At pH 11.0 a dramatic change in active site structure occurs. The five-coordinate high-spin reactive iron(III) changes to a six-coordinate low-spin unreactive iron(III) species. The reason is that the previously vacant distal coordination position of the iron is occupied by a hydroxide ion [8].

The addition of recent crystal structures for several different peroxidases has led to the identification of conserved hydrogen-bonded networks connecting distal and proximal sides [9, 10, 11]. Resonance Raman spectroscopic studies on mutants have shown that these networks can be perturbed [12, 13]. The networks can provide a pathway for electron flow [14], and are represented schematically in Figs. 1 and 2 by the semicircle connecting the distal and proximal imidazole rings. Thus many amino acid residues assist the key catalytic role of the distal imidazole ring.

In Fig. 2, a hydrogen peroxide has diffused into the active site. It is starting to donate a proton to the distal imidazole ring and to form a bond with the iron of the heme. The process is started by the electron-donating properties of the x-nucleophile hydrogen peroxide [3]. Both oxygen atoms have high electron density, and donation of electrons to the iron is assisted by the x oxygen atom, farthest from the iron.

As the hydrogen peroxide binds to the iron, it releases a proton. Transfer of the proton from hydrogen peroxide to the distal histidine residue is only one of several possibilities. There are lone pairs of electrons on water molecules and other residues in the distal cavity. Proton transfer to the distal imidazole occurs in one direction. The simultaneous flow of electrons through the hydrogen-bonded network means that extra electron density is arriving at the key residue from the opposite direction, so the emerging positive charge on the imidazole is largely eliminated (Fig. 2a). Thus the pKₐ value of the distal imidazole is greatly increased. By becoming a much weaker acid the imidazole is able to retain the added proton, even at high pH.

Figure 2b represents the transition state. Binding of the OOH group to the iron is completed, as is binding of the released proton to the distal imidazole. The proton which was transferred to the distal imidazole is poised to be transferred to the x oxygen atom, to form water as the leaving group.

The second half of the reaction is the pathway from the transition state to compound I. This process is started by π back-bonding from the iron to its attached oxygen, which forms the double bond of the Fe⁴⁺ = O group. Removal of electron density from the distal imidazole by the circuit from the distal to the proximal side facilitates proton donation from the imidazole to the x oxygen atom. The arrival of extra electron density at the x oxygen atom, again from the opposite direction to proton addition, further facilitates formation of the water leaving group (Fig. 2c). The imidazole group is restored to its properties as a rather strong acid with a