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Similar enzyme activation and catalysis in hemocyanins and tyrosinases

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Abstract

This review presents the common features and differences of the type 3 copper proteins with respect to their structure and function. In spite of these differences a common mechanism of activation and catalysis seems to have been preserved throughout evolution. In all cases the inactive proenzymes such as tyrosinase and catecholoxidase are activated by removal of an amino acid blocking the entrance channel to the active site. No other modification at the active site seems to be necessary to enable catalytic activity. Hemocyanins, the oxygen carriers in many invertebrates, also behave as silent inactive enzymes and can be activated in the same way. The molecular basis of the catalytic process is presented based on recent crystal structures of tyrosinase and hemocyanin. Minor conformational differences at the active site seem to decide about whether the active site is only able to oxidize diphenols as in catecholoxidase or if it is also able to *o*-hydroxylate monophenols as in tyrosinase.

Keywords: Type 3 copper proteins; Protein crystallography; Hemocyanin; Catecholoxidase; Tyrosinase

Tyrosinase, catecholoxidase, phenoloxidase and hemocyanin all belong to the type 3 copper protein family. Although they share a common active site, they exhibit different functions. While tyrosinase and catecholoxidase are enzymes, hemocyanins are oxygen carrier proteins. The two enzymes, commonly subsumed under the name phenoloxidase, are similar in almost all aspects but different in their functions. Tyrosinase (EC 1.14.18.1) catalyzes the *o*-hydroxylation of monophenols (cresolase or monophenolase activity) and the subsequent oxidation of *o*-diphenols to *o*-quinones (catecholoxidase or diphenolase activity). Catecholoxidase (EC 1.10.3.1), on the other hand, is only able to catalyze the oxidation of diphenols, although it is almost indistinguishable from tyrosinase by sequence and physico-chemical properties other than enzymatic activity (Sanchez-Ferrer et al., 1995; Burmester, 2001).

Phenoloxidases are found in almost all organisms and fulfill several essential biological functions (Sanchez-Ferrer et al., 1995; Sugumaran, 1996; van Gelder et al., 1997; Claus and Decker, 2006; Halaouli et al., 2006; Marusek et al., 2006). They initiate the synthesis of melanin and are responsible for pigmentation of skin and hair, browning of fruit and wound healing in plants and arthropods. Due to the fungistatic, bacteriostatic and antiviral properties of the resulting pigment melanin and its intermediates, phenoloxidases are also key components of the primary immune response of certain invertebrate phyla, especially arthropods (Montefiori and Zhou, 1991; Ashida and Brey, 1995; Johansson and Söderhäll, 1996; Sidibe et al., 1996; Söderhäll and Cerenius, 1998). Furthermore the highly reactive quinones produced by tyrosinase serve to sclerotize the protein matrix of the arthropod cuticle after molting (Andersen et al., 1996; Sugumaran, 1998, 2002). In most arthropods, except chelicerates, phenoloxidase can be found. In crustaceans both, phenoloxidase and hemocyanin, are present at the same time (Aspán et al., 1995; Sritunyalucksana and Söderhäll, 2000; Jaenicke and Decker,

Abbreviations: m-PO, molluscan hemocyanin related phenoloxidase; a-PO, arthropod hemocyanin related phenoloxidase; UV/Vis, ultraviolet/visible (spectroscopy); EPR, electron paramagnetic resonance; FU, functional unit; SDS, sodium dodecyl sulphate; CuA, N-terminal copper site in type 3 copper centers; CuB, C-terminal copper site in type 3 copper centers.

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2003; Terwilliger et al., 2005; Terwilliger and Ryan, 2006). Almost always phenoloxidase can be found in an inactive form, the prophenoloxidase.

Hemocyanins occur in the hemolymph of some species in the phyla arthropoda and mollusca. As extracellular oxygen carriers they are responsible for the precise oxygen delivery from the respiratory organs to tissues. They form very large molecular aggregates with molecular masses up to 8 MDa. Their complex structure enables them to bind oxygen cooperatively with Hill coefficients of up to 7, which are the highest reported for proteins in general. (Loewe, 1978; Decker and Sterner, 1990).

Although a close relationship between phenoloxidase and hemocyanin has been discussed for decades (Lerch, 1994; van Holde and Miller, 1995; Durstewitz and Terwilliger, 1997; Burmester, 2001; Decker, 2005) differences based on sequence comparisons revealed that a common phenoloxidase type does not seem to exist. Also there is no common hemocyanin type. At least two different types of phenoloxidases can be distinguished (van Holde and Miller, 1995; Durstewitz and Terwilliger, 1997; van Gelder et al., 1997; Jaenicke and Decker, 2004). One type (m-PO) is more related to molluscan hemocyanin concerning its general structure and its active site (Fig. 1). The other type (a-PO) is very similar to arthropod hemocyanins and is also found in arthropods together with hemocyanin.

1. Active site

All type 3 copper proteins share a very similar active site although they are different in structure and sequence (van Holde and Miller, 1982, 1995; Salvato and Beltramini, 1990; Burmester, 2001, 2002; van Holde et al., 2001; Jaenicke and Decker, 2004; Decker, 2005; Terwilliger and Ryan, 2006). It is made up by two histidine-coordinated copper atoms. In the oxy form one dioxygen molecule is bound in a side-on bridging coordination between the copper atoms (Fig. 2), as revealed by X-ray crystal structures (Magnus et al., 1994; Cuff et al., 1998; Matoba et al., 2006). Each copper atom is ligated to the protein

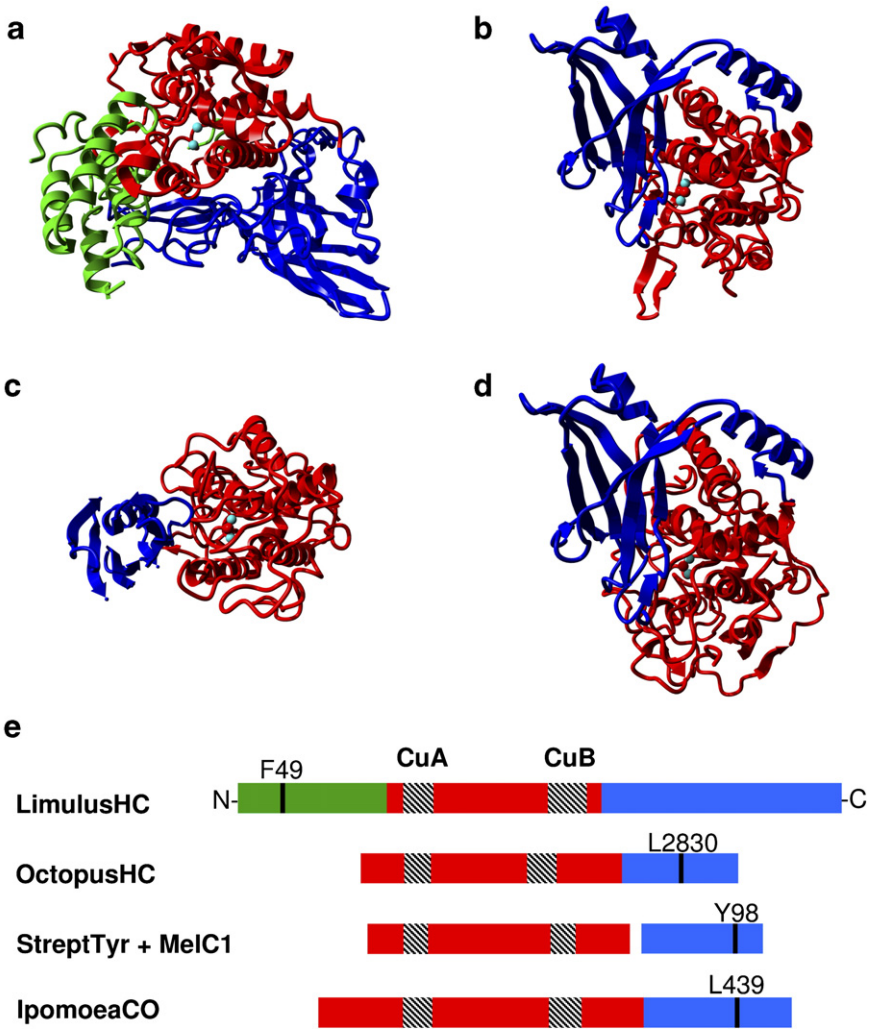

Fig. 1. Arrangement of the domains within the subunit structures of different type 3 copper proteins. (a) *Limulus polyphemus* hemocyanin, (b) *Octopus dofleini* FU g hemocyanin, (c) *Streptomyces castaneoglobisporus* tyrosinase, (d) *Ipomoea batatas* catecholoxidase. (e) Sequence comparison (same color code: domain I (green), domain II (red), domain III (cyan); copper centers are indicated by the hatched blocks, the blocking residues are shown as black bars. In all cases the domains are parts of the subunit with the exception of *Streptomyces* tyrosinase, where an associated caddie protein (MelC1) provides Y98.