Ascorbate oxidase is a multi-copper enzyme that catalyzes the oxidation of ascorbic acid to dehydroascorbic acid. This copper containing blue enzyme is found in cucurbitaceous plants such as pumpkin, cucumber, and melon. It can eliminate ascorbic acid, which has high reducing power in clinical analyses, and detect levels of ascorbic acid. The biological function of ascorbate oxidase is still not clear. One suggestion is that the enzyme participates in a redox system involving ascorbic acids. It may be involved in the reorganization of the cell wall. In pumpkins, ascorbate oxidase expression increased rapidly during growth of callus, development of fruits, and elongation of seedlings. Ascorbate oxidase is present in young and growing tissues of tobacco. It is induced by phytohormone auxin which suggests that it is involved in cell growth. [1]

Ascorbate oxidase consists of three spectroscopically distinct copper centers which comprise of one type I, one type II, and two type III copper atoms. [1] The subunits of 552 residues are each built of three domains arranged sequentially on the polypeptide chain and tightly associated in space. Each subunit consists of four copper atoms bound as mononuclear and trinuclear species, type I and a combination of type II and type III respectively. The type 1 copper atom has two histidine (His), a cysteine (Cys), and an alkyl group (R) as ligands to the central copper atom and is located in domain three. The trinuclear cluster has eight histidine ligands symmetrically supplied from domains one and three and two oxygen atoms either bound as an alcohol (OH) or as a diatomic (O₂). The cluster can be subdivided into two groups, the putative type III copper and the putative spectroscopic type II copper. The type II group consists of a single copper atom with two histidine ligands and an oxygen atom which is trans to the type III copper pair. A pair of copper atoms bounded to six histidines, three ligands on each copper. Bridged by the oxygen atom, the two coppers form the type III copper atom. [2] Images of the mononuclear and trinuclear formations are shown in figures 1 and 2.

Type I Cu centre

image

Figure 1. Type I (blue copper proteins) mononuclear formation. [3]

Type II+III Cu centre image

Figure 2. Trinuclear cluster with type II copper as the lone copper (left) and type III copper as the pair bridged by the alcohol (right). [3]

Symmetry in the type I copper atom is consistent with the Cs point group which consists of the identity operation (E) and horizontal reflection plane (σh). Using bond vector symmetry, an outcome of 3A' + A'' is produced. Examining the point group for Cs shows that both A' and A'' are IR and Raman active and, therefore, in either IR or Raman spectroscopy, four bands should be visible.
A look at the trinuclear cluster produces no symmetry for the group, but each individual copper metal along with its surrounding ligands produce symmetry. The type II copper atom has C$_{2v}$ symmetry with the z-axis going through the Cu-OH bond. Using bond vector symmetry, an outcome of 2A$_1$ + B$_1$ is produced. Examining the point group for C$_{2v}$ shows that both A$_1$ and B$_1$ are IR and Raman active so three bands should be visible in either an IR or Raman spectrum. The two copper atoms in the type III group are equivalent, therefore an examination of one of the copper centers should pertain to both. The geometry of this copper atom is tetrahedral with C$_{3v}$ symmetry and the z-axis through the Cu-OH bond. Bond vector symmetry produces an outcome of 2A$_1$ + E which are both IR and Raman active allowing for three bands to be visible in either IR or Raman.

The enzyme itself appears to have C$_i$ symmetry based on how the image in figure 3 depicts the enzyme.

In various experiments testing ascorbate oxidase distinct spectroscopic bands have been found. Purified ascorbate oxidase has shown a strong absorption band in the ultraviolet region (250 to 300 nm) at 280 nm with a distinct shoulder at 290 nm. The visible light region (300 to 1000 nm) has displayed five absorption bands at 330, 460, 610, 770, and 880 nm. Circular Dichroism (CD) revealed at least four absorption transitions of the copper chromophore in the spectral range of 300 to 700 nm. Ascorbate oxidase exists in complex forms based on the absorption and CD spectra of ascorbate oxidase and comparison with other copper proteins particularly ceruplasmin and laccase, but are distinct from small molecule copper complexes. The absorption and CD spectra of ascorbate oxidase in the range of 400 to 700 nm is similar to the model copper (II) histidine containing peptide complexes. A look at each type of copper group results in type I having high absorption in the visible region, type II has an undetectable absorption, and type III with a strong absorption in the near UV region.

Reference


