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Caeruloplasmin: a plasma protein, enzyme, and antioxidant

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SUMMARY Multifunctional roles of the plasma protein, caeruloplasmin, have been briefly reviewed under three main headings. These are protein functions, enzymic activity, and antioxidant protection. As a plasma protein it is said to play a role in the transport of copper. Since some 95% of serum copper is associated with caeruloplasmin, measurement of the protein provides a useful guide to copper levels. Enzymic functions are related to its oxidase activity. Substrates commonly used for laboratory assay include non-biologically occurring aromatic amines and polyphenols. More recently, a physiological function has been proposed in which the enzyme oxidases iron from the ferrous to ferric state for binding to apotransferrin. This enzymic function of caeruloplasmin has been designated 'ferroxidase'. *In vitro* studies have shown that caeruloplasmin can inhibit the peroxidation of polyunsaturated fatty-acids. This function is in part related to its ferroxidase activity. No antioxidant activity has yet been demonstrated *in vivo* but this possibility is speculatively discussed.

The α_2-globulin fraction of normal human plasma contains a blue-coloured protein, caeruloplasmin, with oxidase activity (Holmberg and Laurell, 1948). Its molecular complexity has led to difficulties in determining its exact molecular weight, and values quoted range from 160 000 to 124 000, with 6 to 8 copper atoms per molecule. A molecular weight of 132 000 with 6 or 7 copper atoms is now widely accepted. Caeruloplasmin, unlike the major intracellular cuproproteins (superoxide dismutases), contains only copper and appears to exist without subunit structure. Fragmentation of the molecule during isolation and purification has led to some confusion concerning subunit structure (Poillon and Bearn, 1966; Ryden, 1971).

Copper-containing and iron-containing metalloenzymes, such as the superoxide dismutases, peroxidases, and catalase, have a common and integrated role in the destruction of toxic forms of oxygen such as superoxide radicals, hydrogen peroxide, singlet oxygen, and hydroxyl radicals (reviewed by Gutteridge (1976)). Even simple copper-amino complexes are themselves catalytically active, possessing superoxide dismutase-like activity (Joester *et al.*, 1972) as well as oxidase activity. Frieden (1974) postulates that throughout biochemistry a close parallelism has evolved between iron and copper metabolism which is of direct relevance to caeruloplasmin function. This hypothesis recognises that the survival of cells in an aerobic environment was followed by the development of iron and copper enzymes such as the cytochromes. As molecular complexity evolved, haem proteins appeared for oxygen transport, followed by iron and copper transport and storage proteins such as ferritin, transferrin, and caeruloplasmin.

The copper in caeruloplasmin has been shown to exist in three distinct states (Vanngard, 1967): type 1 Cu^{2+}, which is responsible for the blue colour absorbing maximally at 610 nm (characteristic of tetrahedral complexes of Cu^{2+} with nitrogenous ligands), type 2, which like type 1 is paramagnetic but colourless, and type 3, which is not paramagnetic but absorbs at 330 nm.

Apart from its important diagnostic value in the genetic disorder, Wilson's disease, caeruloplasmin has received only limited attention in clinical chemistry.

Functions as a plasma transport protein

An important function of caeruloplasmin is said to be that of a copper transporting protein. Some evidence has, however, been obtained by animal studies, which have shown that once caeruloplasmin apoprotein reaches the circulation it is incapable of specific copper binding to form the active caeruloplasmin molecule (Holtzman and Gaumnitz, 1970).

In vitro studies have confirmed, however, that caeruloplasmin can loosely bind divalent transition-metal ions such as zinc, nickel, and cobalt to nonspecific binding sites (McKee and Frieden, 1971) and can exchange its copper for ionic copper in the presence of a reducing substrate at pH 7·4 (Owen, 1975).

The plasma copper can be divided into two main fractions by the strength of copper binding. The non-dialysable fraction is that bound to caeruloplasmin and represents some 95% of the total (Scheinberg and Morell, 1973). Dialysable copper dissociates from complexes with albumin, albumin-histidine, and histidine. These dissociable complexes are thought to be mainly involved in the transport of copper (in the cupric state) from the gut, after absorption, to the liver for incorporation into caeruloplasmin. In the liver, copper is incorporated into the apoprotein before caeruloplasmin is released into the circulation. It is claimed that in the plasma it provides a stable pool of copper for transport and distribution to tissues where it can be incorporated into proteins and enzymes; evidence for this is not entirely conclusive. Once caeruloplasmin has reached its target tissue it is proposed that copper is reduced to the cuprous state before copper release can be effected (Frieden and Hsieh, 1976).

Functions as a plasma oxidase enzyme

Caeruloplasmin was shown by Holmberg and Laurell to have weak oxidase activity towards a variety of polyphenols, aromatic polyamines, and reducing substrates, including ascorbate, hydroxylamine, and thioglycoleate. The use of non-biological substrates, such as *p*-phenylenediamine, gave little support to an 'enzymic role' *in vivo* for the protein and served mainly as a measure of plasma copper levels. The enzyme was often incorrectly referred to as 'copper oxidase'. Detailed studies relating to the oxidase activity of caeruloplasmin have been published by Curzon (Curzon, 1960; Young and Curzon, 1972), who reported that ferrous ions enhanced the activity of caeruloplasmin using *N*,*N*-dimethyl-*p*-phenylenediamine as substrate (Curzon and O'Reilly, 1960). The importance of this finding and its significance were recognised by Frieden and his colleagues, whose work led to the recognition of a possible physiological function for the protein as an enzyme; this was shown to be a ferrous ion oxidising enzyme, which they designated 'ferroxidase' (ferro-O_2-oxidoreductase E.C.1.12.3.1) (Osaki *et al.*, 1966). In order to describe the oxidase activity they classified caeruloplasmin substrates into three main groups

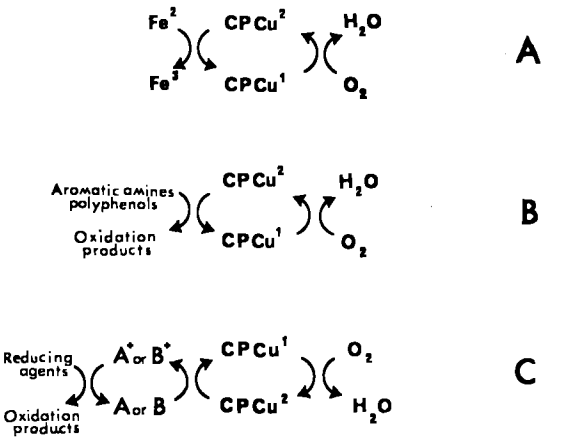

Figure (A) *Ferrous ions (the substrate with the lowest Km);* (B) *Bifunctional aromatic amines and polyphenols which do not depend on traces of iron for their activity;* (C) *Pseudo-substrates comprising various reducing agents (including free-radical intermediates) which can act by way of a group A or B substrate. CP = caeruloplasmin.*

(see Figure) (McDermott *et al.*, 1968; Frieden and Hsieh, 1976). Any reductant can act as a substrate if it can transfer an electron to oxidised-copper-caeruloplasmin without interfering with the autoxidisability of the reduced copper.

FERROXIDASE ACTIVITY AND ITS SIGNIFICANCE

(Group A substrate)

After the classification of caeruloplasmin as a ferroxidase, Frieden and his colleagues proposed a physiological role for the enzyme in the oxidation of ferrous ions (presented to the cell surface) to the ferric state for binding to apotransferrin. This hypothesis was extensively studied *in vivo* using swine made hypocaeruloplasminaemic by copper deprivation (Ragan *et al.*, 1969; Roeser *et al.*, 1970). The animals' cell-to-plasma iron flow became impaired enough to cause hypoferraemia although total body iron stores remained normal. Administration of caeruloplasmin to the animals immediately increased plasma iron, whereas administration of inorganic copper increased plasma iron after the appearance of caeruloplasmin in the plasma. This and other evidence was used to support the role of caeruloplasmin in the movement of iron from cell to plasma. It also provided a link between iron and copper metabolism. Fifty years ago anaemia associated with copper deficiency was described (Hart *et al.*, 1928); its explanation would

seem to fit the present findings. It is likely from the evidence available that ferroxidase provides this link between iron and copper metabolism.

OXIDASE ACTIVITY AND BIOGENIC AMINES
(Group B substrates)
Pharmacologically active compounds, such as the catecholamines, 5-hydroxyindoles, and phenothiazine drugs, are substrates for the oxidase activity of caeruloplasmin in the absence of iron (Lovstad, 1975). It has been suggested that caeruloplasmin may be important in regulating plasma levels of biogenic amines (Barrass and Coult, 1972).

Functions as an antioxidant

IN VITRO ACTIVITY
(Group C substrates)
Antioxidant studies have shown that lipid peroxidation is significantly inhibited by the α_2-globulins of human serum (Placer *et al.*, 1964; Vidláková *et al.*, 1972). Recent work has confirmed and extended these studies to show that the protein mainly responsible for this inhibition is caeruloplasmin (Stocks *et al.*, 1974; Al-Timimi and Dormandy, 1977). The antioxidant activity of caeruloplasmin was expressed as a function of its ability to inhibit the peroxidation of a tissue homogenate. Important naturally occurring catalysts for this type of peroxidation are iron and ascorbate. These are normally compartmentalised within the cell, but after tissue damage or disruption can react together to form a free-radical generating system. As previously mentioned, both iron and ascorbate are substrates for the oxidase activity of caeruloplasmin. Their oxidation results in the inactivation of an important free-radical generating redox couple. Using ox-brain phospholipid liposomes as peroxidisable substrates Gutteridge (1977a), has found that caeruloplasmin antioxidant activity is not only limited to iron-linked catalysis. Both cuprous and cupric ion-catalysed peroxidation was inhibited by caeruloplasmin at a concentration of 300 mg/l in the apparent absence of iron; but caeruloplasmin at a concentration of 10 mg/l showed antioxidant activity towards iron-catalysed but not copper-catalysed peroxidation (Gutteridge, 1977b).

IN VIVO ACTIVITY
(Group A) substrate
Ferrous ions can be oxidised to ferric ions by molecular oxygen. This has been used as an argument against the need for an enzyme to carry out iron oxidation *in vivo* in order that ferric ions may bind to transferrin. The kinetics of ferroxidase and non-enzymic iron oxidation were studied by Osaki *et al.* (1966), who concluded that evidence was strongly in favour of enzymic oxidation. Further support for an enzymic oxidation of iron could be presented from a free-radical point of view. Ferrous ions can form reactive iron-oxygen complexes as well as autoxidise to give 'active oxygens' with damaging properties (for reviews see Gutteridge (1976) and Halliwell (1978)). When

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-} \text{ (superoxide radical)}$$
$$Fe^{2+} + O_2 \rightarrow FeO_2^{2+} \text{ (perferryl ion)}$$

oxidation of ferrous ions is enzymically linked to the copper protein (Figure) complete and rapid reduction of molecular oxygen to water is achieved without apparent free-radical formation. Caeruloplasmin could therefore be described as acting *in vivo* as an antioxidant during iron metabolism.

Caeruloplasmin has been widely used as a measure of 'acute phase reactivity' (Rice, 1961; Crockson *et al.*, 1966). Like other alpha-globulins it tends to be raised whenever there is active tissue damage. During inflammatory states two distinct antioxidant roles could be speculatively ascribed to caeruloplasmin: (1) the prevention of decompartmentalised iron (as a result of tissue damage) acting as a free-radical catalyst; (2) a direct inactivating role against free-radicals, which are produced by phagocytosing white cells, and which may escape into extracellular fluids (these do not contain the protective enzymes catalase and superoxide dismutase). So far caeruloplasmin has been shown to have antioxidant activity only *in vitro* using model membranes and tissue homogenates. Its functions as an antioxidant *in vivo* must remain at present an interesting speculation.

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