Journal of Research and Review in Science Vol. 3, November 2004. pp. 46 - 51

BINDING AND STORAGE PROTEINS FOR ESSENTIAL TRACE ELEMENTS

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ABSTRACT

There is mounting evidence that specific proteins in the body and cellular fluids bind or act as storage molecules for essential trace elements. These proteins play significant roles in the metabolism, storage and transportation of these micronutrients from their sites of absorption in the gastro-intestinal tract to the tissues and cells of the body requiring them for metabolism. The major proteins involved in iron metabolism are ferritin and transferrin. Ferritin is an iron storage molecule while transferring is a transport protein. The proteins associated with zinc metabolism include albumin, metallothionein and cysteine-rich intestinal protein (CRIP). Albumin is mainly a transport protein for zinc in the circulation while metallothionein plays a role in the metabolism and transportation of the micronutrient in the circulation and within the cells. CRIP, however, is probably only a saturable intracellular zinc transport protein.

Caeruloplasmin is a copper transport molecule in the plasma, amd likewise is albumin which serves as a vehicle for its movement to different tissues. Manganese binding proteins have been reported in the mucosa cells of the intestine while selenium containing protein, selenoprotein P, has been identified in the plasma.

INTRODUCTION

The trace elements recognized as essential, for man include iron, zinc, copper, manganese, selenium, molybdenum, iodine, cobalt, fluorine, chromium and nickel. There are good grounds for including vanadium and silicon.

These elements may occur free in circulation and cellular fluids or bound to proteins in these fluids and body tissues. This review focuses on these storage and binding proteins for the essential trace elements. Their known roles are highlighted and discussed in relation to their metabolism, storage, transport and carrier functions. Individual trace elements, in the order listed above are considered along with their binding and/or storage proteins that are understood succinctly to be involved as part of their metabolism in mammalian body. However, those trace elements without known or recognized proteins associated with their metabolism are exempted as the main objective of this article is to put into perspectives the major proteins involved in essential trace elements metabolism.

IRON STORAGE AND TRANSPORT PROTEINS

Iron is essential but its position as a trace element is ambiguous because of the large amounts present in haemoglobin. In terms of its binding and storage proteins, iron can be considered together with these elements. The major storage molecule for iron is ferritin while transferrin is the main transport protein in the serum.

Ferritin

This iron storage molecule has been found in many species from fungi to mammals. It has been extensively studied in rat, horse and man. In these species, it has been isolated from many tissues, including liver, spleen, lung, heart, kidney, placenta, bone-marrow and gastrointestinal mucosa. The metalloprotein has also been found in human and rat serum in concentrations related to body iron stores. Serum ferritin may also be elevated above normal levels in disease states not connected with increased iron loading, including alcoholic liver disease, malignant hepatoma and leukemia (Harrison, 1977).

Ferritin seems to have both protective and reserve functions. A reserve function is required because free iron is toxic and daily losses are small (about 1.0 mg in males and non-menstruating females). The presence of a mobile reserve of iron allows haemoglobin levels to be maintained relatively constant in the face of a fluctuating net supply. Although iron stored as ferritin and haemosiderin is accessible, under normal circumstances only about 1 mg of iron (0.1%) enters and leaves the storage compartment daily in man, mobilization of large amounts of storage iron is a relatively slow process, which may take several days or weeks (Harrison, 1977).

A ferritin molecule is polynuclear iron (hydrous ferric oxide-phosphate) coated by an assembly of protein chains. However, a complete chemistry of ferritin is outside the scope of this review and as such it will not be discussed further here.

Transferrin

Iron is absorbed solely into the blood stream where it is transported bound to a protein known as transferrin. This is a b-globulin protein and normal plasma contains about 2.5g of transferring per litre (Davidson et al., 1975). This gives the plasma a total iron binding capacity (TIBC) of from 45 to 72 mmol/L of iron (250 to 400 mg/100ml). In cases of pregnancy, iron deficiency anaemias and in siderosis, the TIBC is elevated. Decreased values are associated with infections, uraemia, kwashiorkor and haemorrhagic anaemias.
The TIBC is normally fully saturated, and in good health the plasma contains about 100 to 150 mg /100ml. In iron deficiency the level is much lower and the degree of unsaturation very much increased. There exist a number of genetic variants of transferrin. They are useful as genetic markers but each appears to have the same functional capacity.

The transport system has to deal with some 360mmol (20mg) of iron daily liberated from broken – down erythrocytes and also an additional amount of absorbed iron going to and from the cells and the storage depots. The daily turnover of plasma iron is about 630mmol (35 mg). Only a very small proportion of this is at a maximum (Davidson et al., 1975).

ZINC STORAGE AND BINDING PROTEINS

Albumin

This is a protein that is very abundant in the mammalian blood. It serves numerous functions. Among others, it aids in the transportation of many compounds including trace elements such as zinc in the circulation. A normal plasma concentration of Zn is about 12 to 17 mmol/L (800 to 1100 mg/L). most of which is bound to albumin. The concentration of zinc falls, often below 7.5 mmol/L, in conditions where the plasma albumin is low, e.g. cirrhosis of the liver and kwashiorkor (Davidson et al., 1975).

Metallothionein

This metalloprotein was first isolated by Margoshes and Vallee (1957) from equine kidney in their search for biological role of cadmium. This small, cysteine-rich metal-binding protein has since been found in a wide variety of human and animal organs, as well as in most eucaryotic species. It is most abundant in the

parenchymatous tissues of liver, kidney and intestine. The protein has a single polypeptide chain of 61 amino acids, a metal binding capacity of between 5 and 7g atoms /mol and 25-30% of the amino acid residues are cysteine, allowing cross-linking via disulphide bonds. There is no aromatic amino acids in the peptide and the protein exhibits a significant degree of polymorphism with a molecular weight just above 6000. There is sequence homology indicating a highly conserved primary structure.

There are two major isometallothioneins, designated metallothioneins I and II. They differ in amino acid composition and are separable by DEAE ion-exchange chromatography or gel-permeation high performance liquid chromatography (HPLC) (Pulido etal.,1966;Suziki and Maitani, 1981). The asymmetric structure consists of two domains, with differential affinities for various members of bivalent metal ions (Zn, Cu, Co and Ni). The cysteine residues are directly involved in the metal binding. Winge and Miklossy (1982) have demonstrated that the C-terminal domain (a) of mammalian metallothionein binds 4 Zn ions with the help of 22 cysteine sulphydryl groups, while the b domain at the N-terminus only binds 3, with 9 cysteine residues.

All seven Zn ions achieve an approximately tetrahedral geometry of binding as part of oligonuclear clusters in the a or b domains (Vasak and Kagi, 1981). Zinc ions bind preferentially to the a domain. In contrast, the same metallothionein appears to bind 11 or 12 copper atoms, preferentially, adding 6 to the b-domain and 5 or 6 to the a during reconstitution of the apoprotein (Nelson and Winge, 1984).

The fact that metallothionein binds both zinc and copper under physiological conditions suggest that the protein is involved in the metabolism of both nutrient metals (Adekalu, 2003). The metalloprotein has been characterized in the liver, kidney and intestine, and its biosynthesis is controlled by complex processes. It is an inducible protein and under physiological condition, it can be induced by dietary zinc, infection, starvation and stress. It has been induced under experimental conditions by a number of chemicals such as salts of heavy metals and glucocorticoid hormones.

Most mammalian metallothioneins appear to have at least two distinct functional genetic cistrons, expressing two forms of the protein that differ by a few amino acids. The designation of these two categories of metallothioneins is based purely on their elution in ion-exchange DEAE chromatography (Hamer, 1986). The mouse probably has only one functional gene each for metallothioneins I and II, and both are on chromosome 8 (Cox and Palmiter, 1982), only six kilobases apart (Searle et al., 1984). In the human and the horse, one functional gene for metallothionein II and several functional metallothionein I genes (as well as several pseudogenes) have been cloned (Karin and Richards, 1982; Richards et al., 1984). All or most are on chromosome 16 (Schmidt et al., 1984). In other words, metallothionein I appears to have more variants than metallothionein II.

Metallothionein is a fairly rapidly turned over protein. Zinc repletion –depletion experiments with rats demonstrated that 24 hour was sufficient to decrease a substantial pool of metallothionein bound zinc to negligible levels (Richards and Cousins, 1976). The exact functions of metallothionein in intestine, kidney, liver and perhaps other tissues remain to be defined. However, it appears that metallothionein functions as site for temporary storage and detoxification of excess amounts of intracellular copper and zinc, as potential (though probably not crucial) sources of Cu and Zn for other Cu and Zn- dependent proteins within the cell, as a means of shuttling traces of Cu and Zn between kidney and liver and out of the body. Apart from inhibiting intestinal Cu transport in conditions of extremely high zinc intake (where it is induced to high concentrations), it would seem that the role of metallothionein in overall copper and zinc metabolism is otherwise quite passive, adjusting to influx and efflux primarily by displacing zinc in favour of copper which, in turn, may induce more of the protein.

iv. **Cysteine –Rich Intestinal Protein (Crip)**

Cysteine-rich intestinal protein (CRIP) is a 77-amino acid, 8.6 KDa protein with cysteine residues (Birkenmeier and Gordon, 1986). CRIP is minimally expressed at birth and increases to adult levels during the suckling period. The gene is also minimally expressed or not expressed in organs other than the small and large intestine. CRIP has a conserved sequence of amino acids, which includes the cysteine residues, that has been termed the LIM motif for the Lin-II, IsL-1 and Mec-3 genes (Freyd et al., 1990). CRIP is probably a saturable, intracellular zinc transport protein, and metallothionein inhibits zinc absorption by binding zinc in competition with CRIP. The protein may function as an intracellular carrier for zinc in a manner roughly analogous to the role of calbindin in calcium transport (Hempe and Cousins, 1992).

Much remains to be learnt about the biochemistry and molecular biology of CRIP and confirmation of a role for CRIP in zinc absorption or intestinal physiology will require much further research.

COPPER STORAGE AND BINDING PROTEINS

i. Ceruloplasmin

Almost all the copper in the plasma is bound to a specific protein known as caeruloplasmin. As such, very little of this metal is excreted in the urine. The major portion of the dietary copper appears in the faeces. Caeruloplasmin is not normally excreted and this helps to keep the plasma copper fairly constant. The total body copper in an adult is about 2 mmol (100 to 150mg) and there is higher concentration in the liver than in other tissues (Davidson et al., 1976).

ii. **Albumin**

This relatively abundant plasma protein binds and transport numerous essential compounds and trace elements. Apart from caeruloplasmin that binds copper and aids in its transportation to requiring organs of the mammalian body, albumin loosely binds the element and also serves as a vehicle for its movement to different tissues where the metabolism of copper takes place.

MANGANESE BINDING PROTEINS

Up till now, there is no known protein associated with manganese transport and metabolism that has been identified and characterized in the mammalian tissue and circulation. Nonetheless, there is evidence that manganese binds to mucosa cell cytosolic proteins of molecular weights approximately 55, 10, 6.5 kilodaltons and solely to peptides of molecular weight of about 1.0Kda (Adekalu, 2002). The role of manganese binding proteins in the metabolism of the metal will require further research work.

SELENIUM BINDING PROTEINS

Selenium (Se) is a metalloid metal of atomic weight 79, located in group VI of periodic table. Its biochemistry, toxicology and nutritional importance have been reviewed regularly and thoroughly over the last twelve years(Levando, 1985; IPCS, 1987; Lockitch, 1989; Burke, 1993). The element is essential for mammals including humans as a component of two enzymes, gluthathione peroxidase and iodothyronine 5¢- deiodinase. In addition, a distinct selenium containing protein, Selenoprotein P, has been identified in plasma, but its function as yet remains obscure (Arthur and Beckett, 1994; Sheehan and Halls, 1999). There is need for a thorough investigation of the role of selenoprotein P as a practicable aid to determining selenium status.

MOLYBDENUM IN ENZYMES

There is no protein that has been associated with its transportation in the mammalian circulation and cells to date. However, the element forms an essential part of several enzyme systems, e.g. Xanthine oxidase.

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