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The metal affinity of the major myelin basic protein of bovine brain

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This study examined the quantitative aspects of the binding of Cu^{2+} and Zn^{2+} by myelin basic protein of 18500 Da and its components of bovine brain.

Forced dialysis using membrane filters for centrifugal ultrafiltration (VIVASCIENCE) was used to assess the kinetic binding of metal ions.

Gel filtration of metal-saturated MBP eluted with metal free buffer was used to assess the non.equilibrium of metal ions.

Concurrent differential mono- and multiphasic binding patterns resulting from the saturation of whole MBP and its two components I and III with copper ions are strongly suggestive of intermolecular interaction of the charge isomers of whole MBP in binding copper ions.

There was pronounced overall multiphasic binding of zinc ions by any of the proteins tested. It therefore appears that individual MBP isomers may undergo, in addition, intramolecular steric changes thereby recruiting additional histidine residues to form additional metal binding domains.

The saturation curve of the component I with Cu^{2+} was continuous whereas that with Zn^{2+} was biphasic like whole MBP and component III, This may result from metal induced intermolecular interactions of histidine residues of individual MBP components and also suggest that the binding mechanism and binding sites of Cu^{2+} and Zn^{2+} may differ from each other.

For the initial high affinity binding of metal ions by MBP the following results were obtained:

The values of K_D and B_{max} of whole MBP were 2.3×10^{-6} M and 41×10^{-6} M for Cu^{2+} , and 7.2×10^{-6} M and 15×10^{-6} M for Zn^{2+} .

The values of K_D and B_{max} of component I of MBP were 8.8×10^{-6} M and 91×10^{-6} M for Cu^{2+} , and 2.9×10^{-6} M and 14×10^{-6} M for Zn^{2+} .

The values of K_D and B_{max} of component III of MBP were 7.6×10^{-6} M and 36×10^{-6} M for Cu^{2+} , and 19×10^{-6} M and 25×10^{-6} M for Zn^{2+} .

The values of K_D for the initial high affinity binding of copper by components I and III were three to four times as high as those of whole MBP.

Only whole MBP was found to retain both copper and zinc ions following its saturation with metal ions and gel filtration. This feature is suggestive of protein-protein interactions of charge isomers of MBP playing a role in the retention of divalent transition metal ions

The overall metal affinity of MBP was higher for copper than for zinc.

Unlike copper and zinc ions cadmium ions were little retained by whole MBP or its component I on gel filtration, and it was not apparently bound by whole MBP and its component I with forced dialysis.

There was a less basic MBP-like protein associated with MBP of 18.5 kDa that appears to interfere with the metal affinity binding of whole MBP.

The results of this study are further evidence of a possible role of Cu^{2+} and Zn^{2+} in adding to the stability of MBP and its function as an extrinsic protein of the myelin sheath *in vitro*.