REACTIVITY OF ACTIVE CENTRE ANALOGUES OF Cu₂Zn₂ SUPEROXIDE DISMUTASE

Ulrich Weser, Ralf Miesel and Margot Linss

Anorganische Biochemie
Physiologisch-Chemisches Institut der Universität Tübingen
Hoppe-Seyler-Str. 4
7400 Tübingen, FRG

ABSTRACT

Active centre analogues of Cu₂Zn₂ superoxide dismutase were devised and successfully employed. Emphasis was placed on the flexible nature of the superoxide mimicking compounds. Di-Schiff-bases proved most appropriate to fulfil these requirements. Both structural and functional aspects of the copper binding centre of the intact enzyme were met by these complexes. Nanomolar concentrations of copper coordinated in these complexes were sufficient to inhibit the K₃CrO₈ induced chemiluminescence identical to the reaction of Cu₂Zn₂ superoxide dismutase.

INTRODUCTION

There are many small Mr Cu-chelates which are known to exhibit superoxide dismutase like activities (1,2). In these complexes Cu(II) is coordinated in an acetate or biuret type structure. Both geometry and the first shell atoms are unsuitable to keep the transiently formed Cu(I) tenaciously bound to survive competitive biological chelators (3). By way of contrast, in the active centre of Cu₂Zn₂ superoxide dismutase Cu(II) is found in a square planar arrangement and bound to the unsaturated nitrogen atoms of his-44,-46,-61 and -118, respectively. Throughout the catalytic cycle the copper remains firmly bound. The geometry fluctuates from the distorted square planar arranged Cu(II) into tetrahedrally coordinated Cu(I). The flexibility of the protein backbone allows these changes without losing the metal. It was of interest to devise and prepare flexible mononuclear active centre analogues where copper will remain chelated in both the +II and +I form.
DI-SCHIFF-BASE CHELATORS

Convenient chelators encouraged to be di-Schiff-bases prepared from dicarbonyls and a primary amine or by the reaction of an aliphatic di­amine with an aromatic N-heterocyclic aldehyde (4,5). The resulting tetradentate ligand provided 4 unsaturated nitrogen atoms which success­fully coordinated both Cu(II) and Cu(I). As in the case of Cu₂Zn₂ super­oxid dismutase the nitrogen atoms followed the transient conformational changes of the coordination sphere. The aliphatic C₄ unit originating from 1,4-diaminobutane was sufficient to allow these movements (Fig. 1).

Figure 1. Flexible active centre analogue of Cu₂Zn₂ superoxide dismutase

B pyridine or imidazole

MAGNETIC BEHAVIOUR AND ELECTRONIC ABSORPTION

The EPR properties are strikingly similar to those of the native enzyme (Table I). $g_{II}/A_{II}$ an empirical factor is a measure for the degree of tetrahedral distortion (8). Square planar arrangements usually have values between 105 cm and 134 cm. Above 134 cm and up to 250 cm a tetra­hedral distortion can be noticed. The observed 134 cm and 135 cm of the di-Schiff-base-Cu complexes are located at the borderline of such a tetrahedral arrangement and are quite similar to the values obtained with the intact copper proteins.

The less pronounced electronic absorption between 680 and 710 nm was redshifted and can be attributed to d-d transitions in a weak tetra­gonal field. The absorption coefficients ranged between 35 and 150 $M^{-1}cm^{-1}$ (Table II).