

LIPHILIC DERIVATIVES OF Cu,Zn-SUPEROXIDE DISMUTASE: CHARACTERIZATION AND IMMOBILIZATION IN LIPOSOMES

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Introduction

Reactive oxygen species, such as superoxide radicals, play an important role in cellular defense mechanisms. These reactive oxygens also result in oxidative damage of organisms as observed in rheumatoid arthritis. Hence, the oxidative injury of these tissues would be prevented by anti-oxidants or by some enzymes. Cu,Zn-Superoxide Dismutase (SOD) is a very efficient scavenger of oxygen radicals, but the clinical use of this enzyme is severely limited by its short half life in circulation.

Some strategies have been used in the past to increase the therapeutic efficiency of this enzyme in circulation, but with limited success.(1).

Our approach is the incorporation of the enzyme in the lipid matrix of liposomes. We derivatized SOD (Ac-SOD) in order to promote hydrophobic interactions between protein and the lipid bilayers. The localisation of protein in the lipidic matrix is evidenced by the catalytic activity determined before and after disruption of vesicles, compared with identical determinations using non derivatized protein.

This liposomal form of SOD may have a different action in the organism due to:

1. the catalytic action before disruption of vesicles
2. the slow release of the enzyme
3. the increased affinity of the enzyme to biomembranes.

Experimental Methods

Ac-SOD was synthesised by covalently linking fatty acids chains (C8 to C18) to ϵ -NH₂ groups of SOD. The reaction was performed in micellar medium (2).

Solubility of Ac-SOD in water was determined by turbidity measurements.

Partition coefficient was determined in octanol-water mixtures.

The *in vitro* stability of Ac-SOD was performed after incubation in human serum.

Liposomes were prepared by a simplified dehydration-rehydration method (3).

Toxicity studies were performed in Charles River mice.

Results

The increase of the degree of modification as a function of the acyl chloride concentration and of the acyl chain length is shown in FIGURE 1.

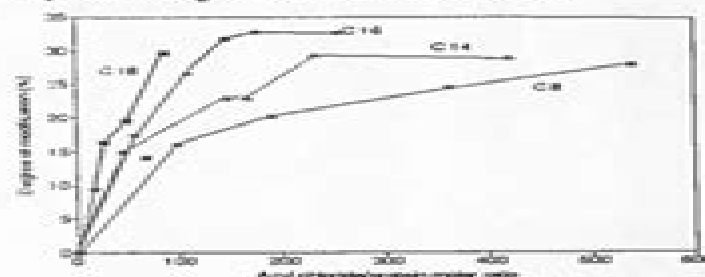


FIGURE 1. Evolution of the degree of modification as function of the acyl chloride/protein molar ratio and of the length of the acyl chain.

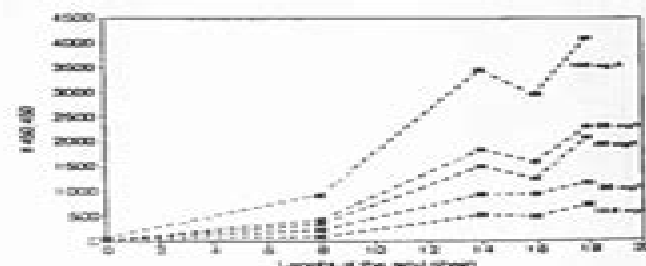


FIGURE 2. Solubility of Ac-SOD in water as a function of the acyl chain, for different enzyme concentrations.

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