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COMPARATIVE STUDIES ON A SUPEROXIDE DISMUTASE EXHIBITING ENZYMATIC ACTIVITY WITH IRON AND MANGANESE AS ACTIVE COFACTOR

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The SOD of *Propionibacterium freudenreichii, ssp. shermanii* belongs to a new group of SOD's capable of retaining activity with either Fe or Mn as active metal cofactor.

Both enzymes exhibit identical secondary structure and immunological determinants. Hydrogen peroxide irreversibly inhibits both enzymes. The protein moiety of the Fe- and Mn-SOD could be digested with trypsin to a single active fragment.

KEY WORDS: Superoxide dismutase, iron, manganese.

INTRODUCTION

All SODs are metalloproteins, containing Cu^{2+} - Zn^{2+}, or Fe^{3+}, or Mn^{3+} as active metal cofactor. The Fe- and Mn-SODs exhibit high structural homology, but activity could only be reconstituted with the metal present in the native enzyme, with 4 exceptions: (a) *Propionibacterium freudenreichii ssp. shermanii*[1] (b) *Bacteroides fragilis*[2,3] (c) *Streptococcus mutans*[4] (d) *Bacteroides thetaiotamicron*[5]

These SODs were reconstitutable with either Fe or Mn and incorporated *in vivo* Mn in the active center, when Fe was absent in the culture medium. The influence of metal exchange on structure and stability, as well as the reason why only these SODs are active with different metal co-factors is unknown.

METHODS

The anaerobic *Propionibacterium freudenreichii ssp. shermanni* was cultured in a complex medium to receive the Fe-SOD, or on an iron free medium to receive the Mn-SOD. Culture conditions and the isolation of the enzyme have been described in detail.[1] Likewise, the methods of presentation of the apo- and reconstituted Fe- or Mn-SOD have been described in detail previously.[1]

Activity was determined by the cytochrome *c* - xanthine oxidase assay[6] or activity staining on polyacrylamide gels.[7]

Spectra were recorded on an Uvikon 820 photometer, a Jasco J-500 A CD-spectrometer and a SFM 23 fluorimeter (Kontron).

To prepare the antibodies against the Fe-SOD, rabbits were immunized by subcutaneous injection of 0.2 mg Fe-SOD, diluted with an equal volume of complete

Freund's adjuvant. Injections were repeated at intervals of 4 weeks until the rabbits were bled.

RESULTS

The secondary structure of the *in vivo* and *in vitro* Fe to Mn exchanged superoxide dismutase was studied by CD-spectroscopy. Both enzymes exhibited an identical β-structure (Figure 1), which transformed into an α-structure upon addition of μmolar concentrations of azide of fluoride (Figure 2). The tryptophan region, studied by CD-, fluorescence-, and UV-spectroscopy, remained unchanged in the presence of theses anions. However the Mn-SOD is more fluorescent than the Fe-SOD, possibly due to metal quenching.

Fused precipitation lines were obtained in the immuno-diffusion-test. This reaction of identity, the common expression of immunological determinants, indicated also a high degree of similarity in the tertiary structure.

The Mn-SOD was less stable towards high temperatures, acid and alkaline pH, and denaturating agents as opposed to the iron enzyme. Azide reversibly inhibited the Fe-SOD to 30% at a concentration of 10 mmol/l, but caused no inhibition of the Mn-SOD, whereas both SODs were insensitive to cyanide and fluoride. Higher concentrations of azide up to 100 mmol/l did not alter the remaining activity. Hyd-

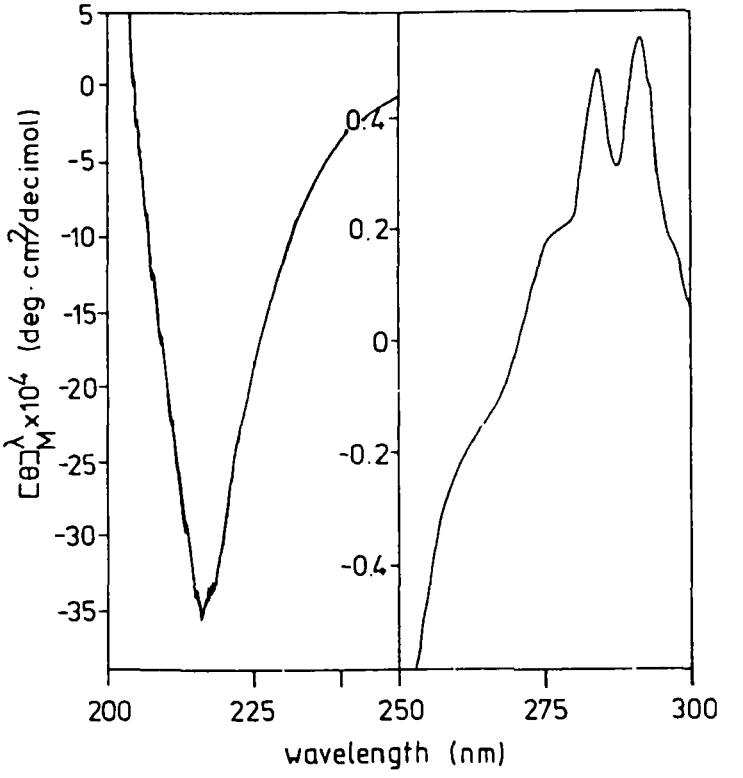

FIGURE 1. CD - spectra of the Fe- and Mn-SOD of Propionibacterium shermanii. CD - spectra were obtained using a protein concentration was 0.6 mg/ml in 50 mmol/l potassiumphosphate buffer pH 7.8.

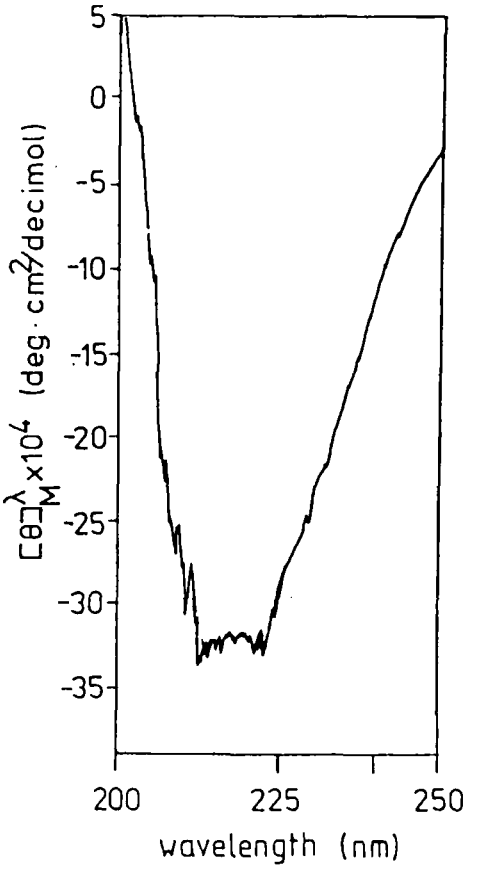

FIGURE 2. CD - spectra in the presence of azide or fluoride CD spectra were obtained as described above but in the presence of azide or fluoride (1 μmol/l).

rogen peroxide (5 mmol/l) irrevisibly inhibited the Fe- and Mn-SOD, whereas 1 mmol/l H_2O_2 was nearly ineffective. The protein moiety of the Fe- and Mn-SOD could be degraded by trypsin to a single fragment without any effect on the enzymatic activities (Figure 3).

DISCUSSION

The SOD of *Propionibacterium shermanni* belongs to a new group of SODs capable to retain activity with different metals as active cofactor.

They exhibit highly similar secondary and tertiary structures, although the Mn- (4 Mn per SOD) content is doubled as opposed to the Fe- content (2 Fe per SOD).

Structural alterations were caused by the addition of anion, without effecting enzymatic activity. Moreover, degration by trypsin decreased the protein moiety, but was also without effect on the enzymatic activity of the SOD. Structural analyses of this minor active part may clarify why these SODs are active with different metals.

Hydrogen peroxide, the product of the enzymatic reaction, is reported to inactivate

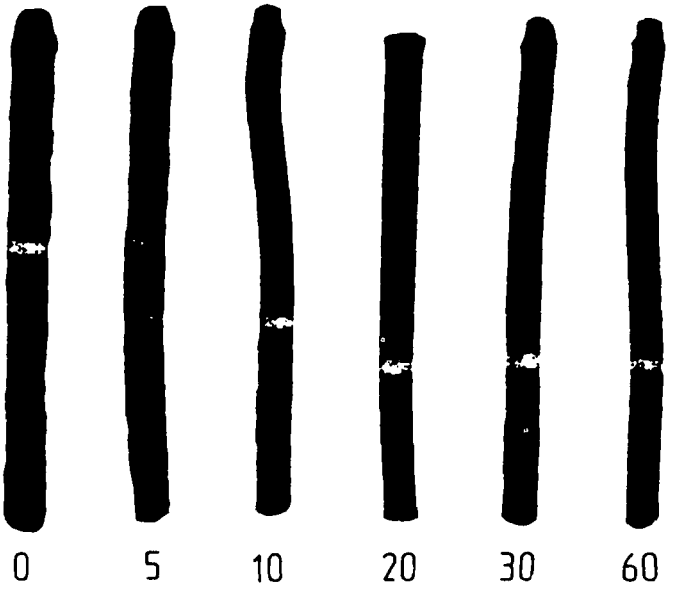

FIGURE 3. Activity staining of the Fe-SOD after treatment with Trypsin Fe-SOD (1 g/l) was incubated with 10 mg/l trypsin in 50 mmol/l potassiumphosphate buffer pH 7.4 and the reaction was stoped by the addition of 10 mg/l trypsin inhibitor. Electrophoresis was performed on 7% polyacrylamide gels and activity staining was performed according to.[7]

specifically the Cu-Zn- and Fe-SODs, whereas the Mn-SODs are resistant towards this agent. The inactivation by H_2O_2 was even used to identify SODs in the crude extracts of bacteria. As H_2O_2 irreversibly inhibits the Fe- and Mn-SOD, these tests may lead to errors when used to distinguish between Fe- or Mn-SODs in bacterial lysates.

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