〔Free Rad. Biol. Med., **13**, 205-210 (1992)〕 [Lab. of Pharmaceutics]

The Site of Nonenzymic Glycation of Human Extracellular-Superoxide Dismutase *in vitro*.

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Nonenzymic glycation of EC-SOD, both *in vivo* and *in vitro*, is associated with a reduction in heparin affinity, whereas the enzymic activity is not affected. The glycation sites in EC-SOD are further studied in the present article. From a chymotryptic digest of *in vitro* glycated EC-SOD, two peptides with affinity for boronate could be isolated. Amino acid sequence analysis showed that both encompassed the carboxyterminal end. ε-Glucitol lysine was identified at positions 211 and 212. The primary glycation sites in EC-SOD are thus lysine-211 and lysine-212 in the putative heparin-binding domain in the carboxyterminal end.

〔Arch. Biochem. Biophys., **297**, 155-161 (1992)〕 [Lab. of Pharmaceutics]

The Heparin Binding Site of Human Extracellular-Superoxide Dismutase.

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EC-SOD in plasma is heterogeneous with regard to heparin affinity and can be divided into three fractions. It appeared that this heterogeneity is not dependent on the carbohydrate structure. Recombinant EC-SOD C treated with trypsin or endoproteinase Lys C, which lost three lysine residues (Lys-211, Lys-212, and Lys-220) or one lysine residue (Lys-220) at the C-terminal end, had no or weak affinity for the heparin HPLC column, respectively. The proteinase-treated r-EC-SOD C also lost triple arginine residues which are adjacent to double lysine residues. These results suggest that the heparin-binding site may occur on a "cluster" of basic amino acids at the C-terminal end of EC-SOD C.

〔Thromb. Haemostas., **68**, 464-469 (1992)〕 [Lab. of Pharmaceutics]

The Interaction of Botrocetin with Normal or Variant von Willebrand Factor (Types IIA and IIB) and Its Inhibition by Monoclonal Antibodies that Block Receptor Binding.

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Two-chain botrocetin binds to vWF from plasmas of type IIA or IIB von Willebrand disease and its interaction is indistinguishable from that with normal vWF. However, an activated complex formed between botrocetin and IIB vWF expresses an enhanced biological activity for binding to GPIb whereas the complex with IIA vWF has a decreased binding activity. Two anti-vWF monoclonal antibodies abolished direct binding between botrocetin and vWF. This suggests that they recognize an epitope(s) on the vWF molecule in close proximity to the botrocetin binding site.