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Design, solid phase synthesis and evaluation of cationic ferrocenoyl peptide bioconjugates as potential antioxidant enzyme mimics

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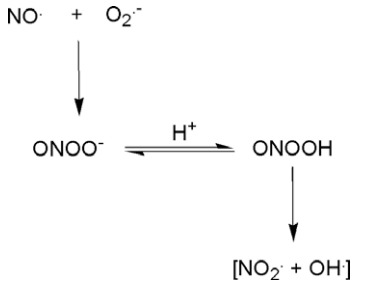
ABSTRACT

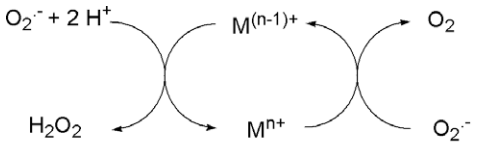
Synthetic C-terminal amidated cationic ferrocenoyl peptide bioconjugates Fc-Orn-Orn-Orn (**1**) and Fc-Tyr-Orn-Orn-Orn (**2**) were rationally designed as superoxide dismutase (SOD) mimics based on the structure of the iron SOD from *Escherichia coli*. Ferrocenoyl peptide bioconjugates **1**, **2** and ferrrocenecarboxylic acid (**4**) were subsequently evaluated as SOD mimics and as inhibitors of peroxynitrite-mediated tyrosine nitration. Due to their cationic character, ferrocenoyl peptide bioconjugates **1** and **2** exerted an acceptable SOD activity (EC_{50} = 575 μM and 310 μM, respectively) in comparison with **4** (EC_{50} = 1.4 mM). The C-terminal amidated cationic peptide Ac-Tyr-Orn-Orn-Orn (**3**), designed as marker of peroxynitrite, was used to evaluate the inhibitory activity of **1** and **4** towards peroxynitrite-mediated tyrosine nitration. Both compounds proved to inhibit the nitration especially the cationic ferrocenoyl peptide bioconjugates **1**. The ferrocene moiety of conjugate **2** displayed a strong inhibitory activity of peroxynitrite-mediated nitration of the neighboring tyrosine.

The combination of superoxide anion ($O_2^{-\cdot}$) and nitric oxide ($NO^{\cdot}$) at diffusion-controlled rates results in the formation of peroxynitrite ($ONOO^-$), a toxic and powerful oxidant that provokes cellular damages.[1,2] Indeed, the peroxynitrous acid, the conjugate acid of $ONOO^-$ (pK_a = 6.8) leads to the release of $NO_2^{\cdot}$ and $OH^{\cdot}$ radicals through homolytic cleavage (Scheme 1).[3–5] These radicals are particularly involved in the oxidation and the nitration of DNA, proteins and lipids.[6] The main effects of peroxynitrite on proteins are the nitration and the hydroxylation of tyrosine and tryptophane residues, the formation of dityrosine and the modification of sulfur containing residues.[7,8]

During the last decade, antioxidant enzymes such as peroxidase, reductase and selenoproteins[9–11] have emerged as relevant species preventing the oxidative action of peroxynitrite. Among these species, metallo-enzymes superoxide dismutases (SODs) that inhibit the peroxynitrite formation by detoxifying cells from $O_2^{\cdot-}$ and thus subtracting the radical to its reaction with nitric oxide[12] have been extensively studied. These enzymes are dimeric or tetrameric and



Scheme 1. Mechanism of formation of $NO_2^{\cdot}$ and $OH^{\cdot}$ from the combination of nitric oxide and superoxide.



M = Cu, Fe or Mn and n = 2 (Cu) ou 3 (Fe, Mn)

Scheme 2. Cyclic mechanism of metallo superoxide dismutases.

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exhibit in their active site a coordinated metal atom involved in the dismutation catalysis of $O_2^{\cdot-}$ into water and molecular oxygen according to a cyclic mechanism (Scheme 2).[13,14] In addition, SODs are also known as peroxynitrite scavengers because they catalyze the decomposition of peroxynitrite by nitrating their own tyrosine residues, or tyrosine residues of other proteins.[15–17]

The defensive role of SODs is now well established in several pathologies such as degenerative diseases or ischemia–reperfusion injury.[10,11] Therefore, the design, the synthesis and the evaluation of antioxidant enzyme mimics for potential therapeutic applications has recently received a significant interest.[18–21] Among the potential SOD mimics, metalloporphyrin derived compounds and nitrogenated metalloheterocycle complexes have been particularly studied.[22–24] Some of these metallocomplexes have also been shown to inhibit the peroxynitrite-mediated tyrosine nitration.[9,25] Polyphenols such as gallic acid and catechin have been reported to exhibit this activity.[26–28]

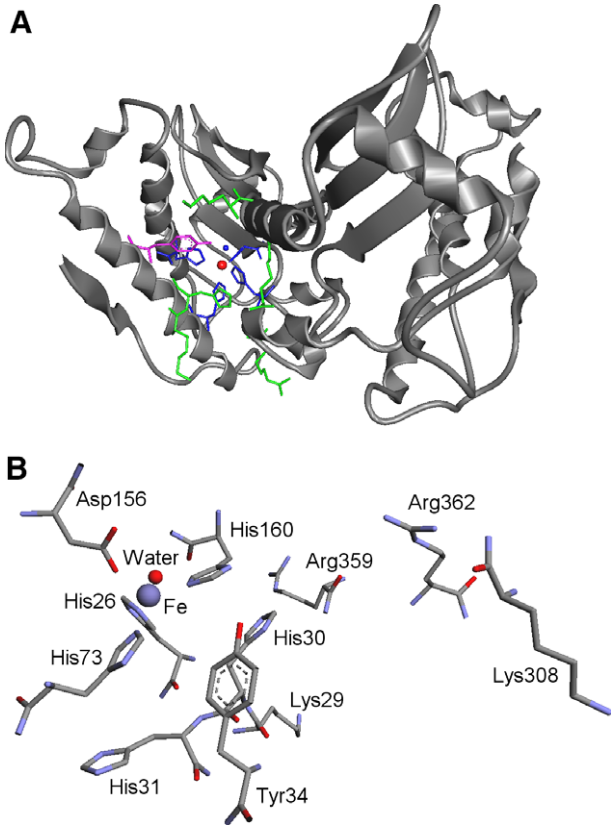
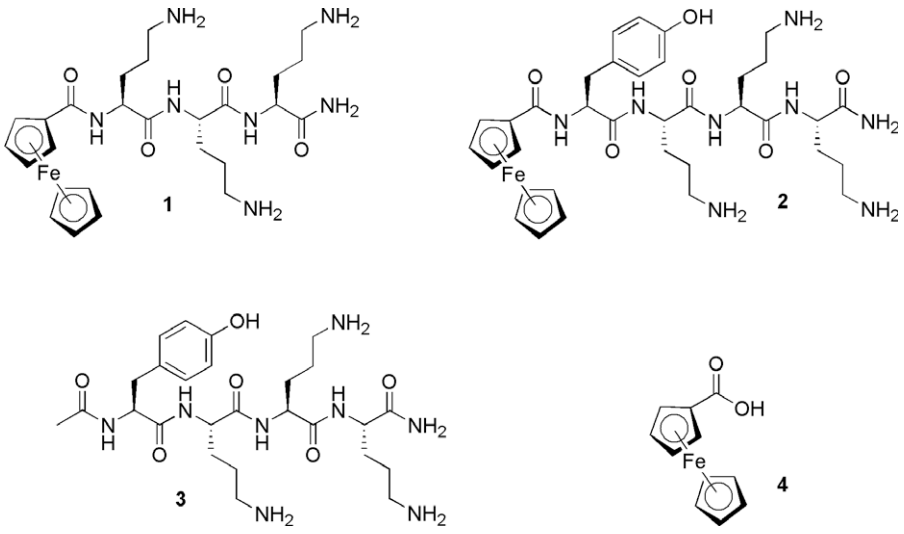


Figure 1. (A) Tri-dimensional structure of Fe-SOD with α-helixes and β-sheets forming a funnel as access to the metal of the active site with positively charged residues (in green) and residues coordinated with the metal (in blue). (B) Overview of the electrostatic funnel as access to the metal.

Ferrocene-derived compounds display a broad range of biological activities.[29,30] Particularly, ferrocene containing peptide conjugates have been synthesised and evaluated for their antiproliferative activity against human leukemia cells.[31] The design and the synthesis of cationic ferrocenoyl peptide bioconjugates have been reported as antibacterial agents.[32] However, although metallocenes have been suggested as SOD mimics,[33] to the best of our knowledge, no study has been reported so far. Aiming at the development of new antioxidant enzyme mimics based on the structure of the iron SOD (Fe-SOD) from *Escherichia* coli (pdb code 1ISA) (Fig. 1), the synthesis of ferrocenoyl peptide bioconjugates and their evaluation as SOD mimics and inhibitors of peroxynitrite-mediated tyrosine nitration were investigated.

Fe-SOD is a homodimeric protein with predominant α-helices. The interface between the two subunits forms a funnel like access to the active site constituted by the metal center, an atom of iron coordinated by three histidine residues and a molecule of water (colored in blue in Figure 1). This funnel embodies positively charged residues (histidine, arginine and lysine residues colored in green in Figure 1) responsible for the electrostatic guidance of the substrate. The tyrosine residue Tyr34 (colored in pink in Figure 1) has also been identified as an important residue due to its strong reactivity with the peroxynitrite anion leading to the corresponding nitrated tyrosine.

Based on these observations, cationic ferrocene-peptide bioconjugates Fc-Orn-Orn-Orn (**1**) and Fc-Tyr-Orn-Orn-Orn (**2**) were designed (Scheme 3). In order to exert an attractive effect towards the two anions $O_2^{\cdot-}$ and $ONOO^-$, a cationic tripeptide sequence Orn-Orn-Orn was introduced on bioconjugates **1** and **2**. Ornithine (pK_a δ-$NH_2 \approx 10.5$) was preferred over other natural cationic amino acids to prevent biodegradation during potential in vivo applications. A tyrosine residue was introduced on peptide **2** as potential nitration site and a ferrocene moiety was placed next to the tyrosine residue to evaluate the effect of the metallocene moiety on the peroxynitrite-mediated tyrosine nitration. In both cases, the ferrocene moiety was anchored to the N-terminal via an amide



Scheme 3. Structures of ferrocenoyl peptide conjugates **1** and **2**, peptide **3** and ferrocenecarboxylic acid **4**.