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“Structural characterization of heme-protein models”

Introduction:

Heme-proteins are a family of important macromolecules ubiquitous in biological systems and characterized by a shared prosthetic group, the “heme” group: a tetrapyrrole macrocycle (the porphyrin), which bind an iron ion. They perform numerous biological functions, and are a clear example of the fine modulation of the metal center properties by the protein environment. A powerful approach for deeply understand the factors which influences the properties of natural heme-proteins is the development of artificial peptide-based models that reproduce active sites in simplified frameworks. In this field, the “Artificial Metalloenzyme" group in Naples developed a family of heme mimetics named Mimochromes.[1,2] These molecules have a sandwich like structure, in which a porphyrin is embraced between two helical peptides covalently linked to deuteroporphyrin IX (DPIX) through amide bonds between the heme propionic groups and the ε-amino groups of two lysine residues on the peptide chains.
The last achievement in the Mimochrome family was the design of molecules in which the heme has mono-hisitidine coordination, which have peroxidase like activity (mimochrome VI and its analogues).[3,4] The catalytic performance of these molecules was increased by using a re-design procedure. However, due to lack of information on the three-dimensional structures of mimochrome VI and its successors, it was not possible to outlain the analysis of structure-activity relationship.

Aim:

During this project the research activity was pointed on the structural characterization of the mono-histidine mimochrome models. In fact, the main drawback of these molecules relies in the lack of evidence on their structures. In fact, the attempts to solve the three dimensional structure were unsuccessful, as the analysis of the Nuclear Magnetic Resonance (NMR) spectrum of Co(III)-Mimochrome VI revealed the presence of different species in solution. Indeed, the engineering of peptide-based heme containing catalysts that fold in a unique and well-defined conformation is an ambitious goal, since the strategies used for the molecular design should take into account all the variables needed to specify both the structure and the function. With the aim of stabilizing the peptides secondary structure and overcoming the multiple species existence problem, a well-established helix stabilizing non-coded amino acid (α-aminoisobutyric acid, Aib (U)) has been inserted. The new molecule has been named Mimochrome VIa. Subsequently, the lysine involved in the amide bond formation between the porphyrin and the non coordinating peptide chain has been replaced with 2,4-Diaminobutyric Acid. The new molecule was named Lys^{9}Dab(D)-

Mimomochrome VIa. Here, the structural characterization of these new models has been undertaken.

Research activity:

Mimochrome VIa was synthesized as previously described for mimochrome VI. However, when the Co(III)- Mimochrome VIa complex was analyzed by RP-HPLC, the chromatographic profile, shown in **Fig. 1**, evidences the presence of four peaks. The four species were separated by preparative HPLC and analyzed. The ESI-Mass spectra are in agreement with the mass expected for Co(III)-mimochrome VIa, for all the species.

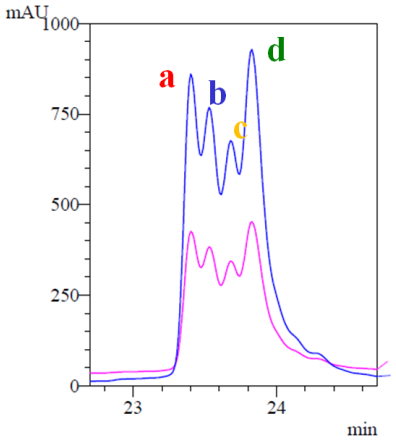

Fig. 1 RP-HPLC chromatogram of Co(III)- Mimochrome VIa.

Here, the analysis by Nuclear Magnetic Resonance of the first species (**peak a**) in H_2O/TFE 60/40 (v/v) at 8°C has been undertaken (1H 1D NMR spectrum is reported in **Fig. 2**). Resonances assignment was accomplished by 2D experiments (NOESY, TOCSY, DQF-COSY) by means of the sequential technique for both deuteroporphyrin and peptide protons.

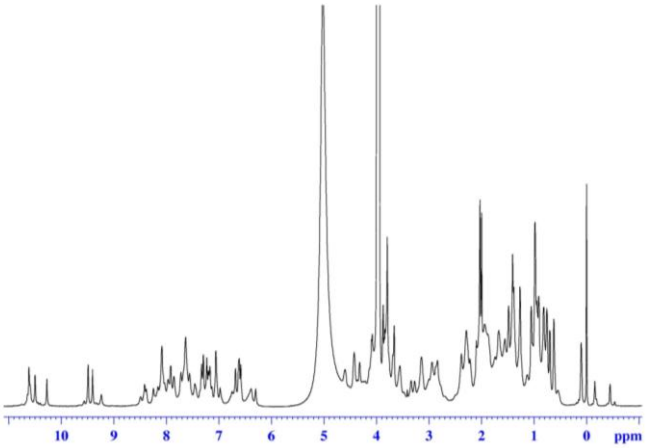

Fig. 2 1H NMR spectrum of Co(III)-mimochrome VIa in H_2O/TFE 60/40 (v/v) at 8°.

The NOE contacts between Lys^9 sidechain and propionic α,α'- and β,β'-CH_2 protons were crucial to identify the tetradecapeptide chain as the one bound to propionyl group in position 18, and the decapeptide chain the one linked to the propionyl group at position 2. Chemical shifts of peptide chains show αCH resonances significantly up-field shifted, relative to their random coil values (**Fig. 3a**). Indeed, the pattern of the NOE connectivities strongly supports the presence of a helical structure of the peptide chains (data not showed).

Imidazole δ-CH and ε-CH protons have different connectivities with the DPIX protons, thus permitting to determine the histidine orientation with respect to the porphyrin plane. The imidazole ring presumably lies in a plane that is orthogonal to the DPIX plane and oriented almost parallel to the C_1-C_{11} direction (**Fig. 3b**). Several NOE contacts were also observed between the porphyrin protons and key residues on the peptides: Leu^2 and Leu^5 in the teradecapeptide, Aib^3 and Aib^7 in the decapeptide, indicating that the peptides are facing the porphyrin plane, forming with it hydrophobic interactions. On the basis of these observations, the determination of the Co(III)-Mimochrome VIa three dimensional structure is now under course.

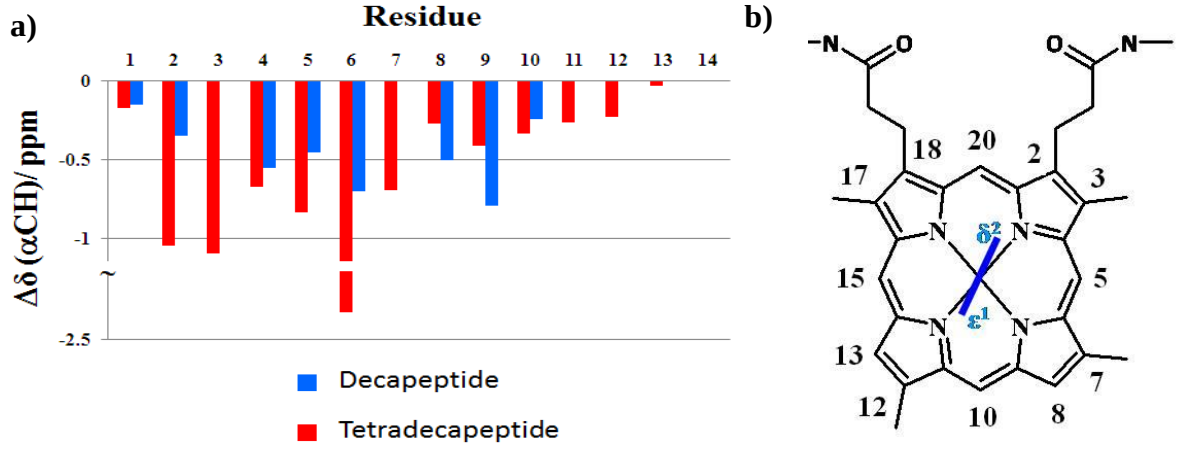

Fig. 3 a) Chemical shift index Δδ(αCH) (δ_{osb} - $\delta_{randomcoil}$) of Co(III)- Mimochrome VIa peak a; b) orientation of the hidtidine imidazole ring with respect to the DP IX plane.

Paramagnetic NMR of artificial peroxidase models

During this research project, I spend two month working at the Instituto de Tecnologia Química e Biológica António Xavier of the Universidade Nova de Lisboa (ITQB), in the Inorganic Biochemistry and NMR group, under the supervision of Prof. R.O. Louro. Here, we used a methodology based on the measure of the paramagnetic NMR shifts in order to determine the histidine orientation in a five-coordinated mimochrome model: Lys^9Dab(D)-Mimomochrome VIa.[5,6] This molecule was chosen, as it showed attractive functional properties, been the model characterized by the higher peroxidase activity between the mimochrome family.
The Lys^9Dab(D)-Mimomochrome VIa Iron(III) complex is in high spin state in aqueous solutions and in mixed water– trifluoroethanol solvent (at pH ~ 6), as confirmed by both UV-vis spectroscopy and NMR. In order to simplify the NMR characterization, we set out to induce the formation of a low spin species. Therefore, we analyzed the metal binding properties. The ligand concentration at which saturation occurs was determined. Then, the NMR spectra of Lys^9Dab(D)-Mimomochrome VIa with 0.8 M NaN_3 and 20 Mm KCN were collected in phosphate buffer pH 5.8 60mM/TFE 60/40. The 1D proton NMR spectrum in the presence of azide shows two set of signals, at around 50 ppm (high spin region) and at around 35 ppm (low spin region). On the contrary, the Fe(III)-Lys^9(D)MimomochromeVIa 1D proton NMR spectrum at 25 °C in the presence of 20 mM KCN appear typical of low spin Iron (III) heme complexes (**Fig. 4**). Cross peaks were observed in the 2D NOESY spectrum, and we were able to assign the ^{1}H shifts of the porphyrin methyls. In order to assign the ^{13}C resonance of the porphyrin methyls, the Heteronuclear Multiple Quantum Coherence (HMQC) 1H-13C NMR spectrum was collected. This information can be used in order to establish the histidine orientation with respect to the porphyrin plane. The analysis of the data is currently under course.

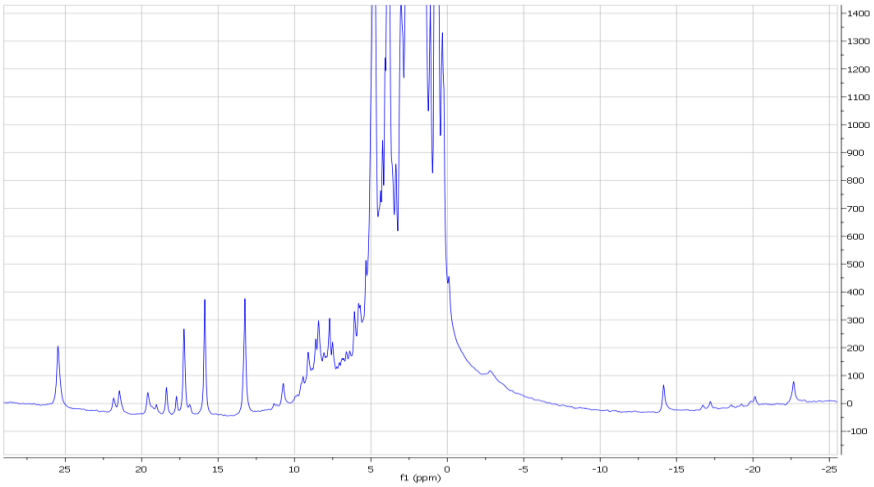

Fig. 4 1D 1H NMR spectrum of Fe(III)-Lys^{9}Dab(D)-Mimomochrome VIa in the presence of 20 mM KCN in phosphate buffer 60 mM pH 5.8 /TFE 60/40.

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