A site-directed mutagenesis study to assess the mechanism of superoxide scavenging by *Archaeoglobus fulgidus* Neelaredoxin

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Neelaredoxin (Nlr) is a 125 amino-acid blue coloured protein, containing a single iron atom/molecule, which in the oxidised state is high spin ferric. Nlr is the main protein from *A. fulgidus* which is reactive towards $O_2^{\cdot-}$. Nlr is able to both reduce and dismutate $O_2^{\cdot-}$, thus being a bifunctional enzyme. Kinetic and spectroscopic studies indicate that Nlr's superoxide reductase activity may allow the cell to quickly eliminate $O_2^{\cdot-}$, in a NAD(P)H dependent pathway. On the other hand, Nlr's superoxide dismutation activity will allow the cell to detoxify $O_2^{\cdot-}$ independently of the cell redox status [1].

The mechanism by which Nlr reacts with superoxide was assigned by pulse radiolysis. The role of the putative iron sixth ligand [2], E-12, in the reactivity of Nlr was assessed by studying two site directed mutants [3]: E12Q and E12V. Our results show that the E12Q mutant has a mechanism very similar to the wild-type enzyme, while the E12V mutant show considerable differences, in particular during the superoxide reduction step. These results together with our calculations for the electrostatic surface around the iron center, obtained by molecular modeling, led us to propose a role for the E-12, D-8 and K-13 residues in the reactional mechanism of Neelaredoxin [3].

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Dinuclear copper(II)pyrazolate complexes as model system for the catechol oxidase metalloenzyme - correlation of structure and catalytic activity

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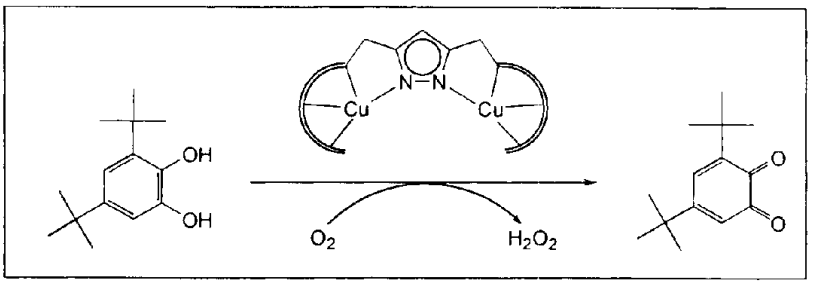
Several new dinuclear copper(II)complexes based on pyrazolate ligands bearing chelating side arms of varying length and number were synthesized and structurally characterized. Depending on the ligand scaffold, the Cu···Cu separation can be tuned in the range 3.5 to 4.5 Å. The catalytic activity of the novel complexes for the oxidation of 3,5-di-*tert*-butylcatechol was investigated. The results suggest a correlation of the Cu···Cu distance and the catalytic efficiency of the particular complex, where the shortest Cu···Cu distance seems to be particularly favorable for catecholase activity. In order to gain further information on possible modes of substrate coordination, adducts with the substrate mimic tetrachlorocatechol were synthesized and characterized by x-ray crystallography in two cases. Formation of H_2O_2 as the product of O_2 reduction was confirmed experi-mentally, and a possible mechanism of the catecholase reaction of the present complexes is proposed.



Modelling biological electron transfer: A heterodimeric redox maquette

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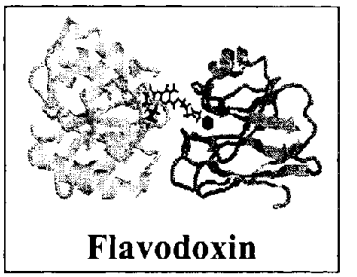

We have constructed a maquette of two redox proteins as a manipulable system for modelling biological electron transfer. The redox active centres of the FMN-containing flavodoxin (shown on the left of the figure in light grey) and the blue copper protein azurin (on the right in dark grey) have been directly linked by a bifunctional organic spacer molecule, shown in the figure in stick form coordinating the copper atom of azurin. This linker molecule, 8α-imidazolyl-N-propylyl-amino-riboflavin, or IPAR, has been shown to reconstitute apoflavodoxin, while its imidazole moiety can in turn associate to the exposed copper site of the His117Gly mutant of azurin that we use. Using laser flash photolysis combined with the reductive action of deazariboflavin we were able to characterise the electron transfer along the linker from the reduced flavodoxin to the oxidised azurin. This reaction was shown *not* to be dependent on protein concentration and had a rate of $14\ s^{-1}$. The model system can now be extended to using various linkers and substituting other proteins, thus allowing us to investigate the effects of different factors such as redox potential and distance on the rate of electron transfer within the complex.

Synthesis and crystal structure of cyclometalated ruthenium(II) complexes for bioapplications

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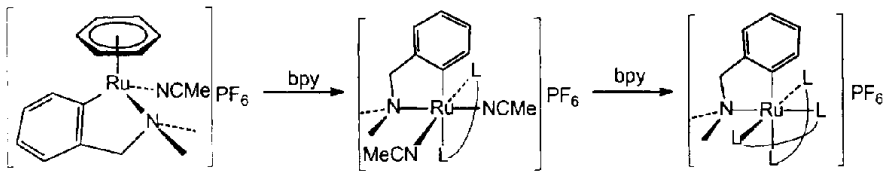
The synthesis of a series of ruthenacycles shown in the Scheme is described. Complex **1** was characterized by an X-ray



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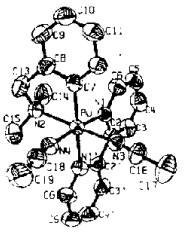

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crystallography. Formation of "decapped" species **1** was shown to be due to the light-induced dissociation of η^6-bound benzene. Complex **1** with two labile acetonitrile ligands is a convenient precursor for syntheses of a variety of cycloruthenated compounds with three different bidentate ligands. The synthetic strategy outlined in the Scheme demonstrates a mechanism for tuning the redox potentials of the ruthenium(II) centers by introducing different LL ligands. This is very urgent from the point of view of designing organometallic mediators for the electron transport between an electrode and active sites of redox enzymes.

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Developmen of an electrochemical biosensor for nitrite determination

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Nitrites are widely used in agricultural and technological practices and may occur naturally in biological materials. The high toxicity of these substances aroused a great deal of interest on their determination. A large number of nitrite determination methods have been reported but, although reliable, many of them are lengthy or lack specificity and/or sensitivity.[1]

Cytochrome *c* nitrite reductase (NiR), isolated from *Desulfovibrio desulfuricans* ATCC 27774 membranes, catalyses the direct reduction of nitrite to ammonia. It is highly stable and readily isolated in significant amounts. Earlier cyclic voltammetry experiments showed an electrocatalytic response of NiR in the presence of its substrate, which in combination with its specificity and stability, turn the enzyme in an attractive candidate for the construction of an electrochemical biosensor for nitrite determination in complex samples. Attempts to design a NiR-based electrode were already made but the stability of the electrode-enzyme link was difficult to obtain.[1,2] In this study, we propose the NiR immobilization on a glassy carbon electrode surface, by co-deposition with Nafion®, a perfluorinated cationic-exchange polymer, which is able to accumulate the chemical mediator (methyl viologen) in the film. This system permits a reagentless analysis of nitrite. Upon nitrite addition, the catalytic current increased linearly between a large dynamic range (0.2 to 2.5mM). There were no interferences from sulfite or nitrate. The electrode is active for 2 days, when stored at 4°C.

We acknowledge the Fundação para a Ciência e a Tecnologia for the financial.

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Iodination of L-tyrosine by vanadium-dependent haloperoxidases

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Marine organisms produce an enormous array of halometabolites, most of them involved in chemical defenses roles. Iodometabolites are quite common in marine environment, especially in seaweeds, where monoiodotyrosine (MIT) and diiodotyrosine (DIT), a well known intermediates in the biosynthesis of vertebrate's hormone thyroxin, were detected [1,2]. The role that these iodinated compounds could perform in these organisms remains unknown and since vanadium-dependent haloperoxidases (V-HPO) isolated from brown seaweeds catalyse iodination and bromination reactions[3], we decided to investigate the possible role of V-HPO in tyrosine iodinated derivatives formation.

The iodination of L-tyrosine by hydrogen peroxide and iodide catalysed by the vanadium-dependent haloperoxidase (VHPO) from the seaweed *Laminaria saccharina* was studied. The reaction was followed by the formation at 290 nm of MIT. The reaction presents a Michaelis-Menten behaviour over the conditions tested. Inhibition by hydrogen peroxide was observed. Kinetic parameters were determined and the type of mechanism involved is discussed.

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Synthesis and characterization of cis-Pt(ii)Cl_2 complexes with mixed aliphatic amines. Comparison of the biological activity with their corresponding *trans* isomers

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The design of platinum anticancer drugs has recently moved from the early empirical structure-activity relationships, and efforts have been directed toward the synthesis of unconventional compounds mechanistically different from cisplatin (1). Among them, *trans*-platinum (II) complexes with mixed aliphatic amine ligands, have shown promising results in *in vitro* assays (2). We are recently studied several *trans*-[$PtCl_2LL'$] complexes where L= dimethylamine, propylamine and butylamine and L'= isopropylamine (3). The analysis of the cytotoxic properties of these compounds against ovarian tumor cell lines sensitive and resistant to cisplatin indicate that these complexes have a cytotoxic activity (IC_{50} values) similar to that of cisplatin in tumor cell lines sensitive to this drug (Pam 212 and CH1) and, interestingly, they are able to circumvent cisplatin resistance in Pam 212-*ras* overexpresing H-ras oncogenes (IC_{50} values between 6 μM and 21 μM, being the IC_{50} of cisplatin 156 μM). In order to complete our study we have look at the corresponding "cis" analogues. Thus, we have synthesized several *cis*-[$PtCl_2LL'$] complexes where L= methyl, dimethylamine, propylamine and butylamine and L'= isopropylamine. The synthesis (4) involves obtaining the iodine dimer with isopropylamine and the subsequent breaking of the bridge with the different aliphatic amines. These complexes have been characterized by standard techniques such as elemental analysis, IR and NMR spectroscopies of proton, carbon and platinum. The analysis of the cytotoxic properties of these compounds indicate that only *cis*-[$PtCl_2$(isopropylamine)(methylamine)] posseses activity in the cisplatin-sensitive line CH1 showing an IC_{50} value of 69.5 μM. However, the IC_{50} of cisplatin is 3.8-times lower (18μM). Thus, altogether the results show that the *trans*-Pt compounds with mixed aliphatic amines are endowed with better cytotoxic properties than their *cis* counterparts being, in addition, able to overcome cisplatin resistance.

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Structure-function studies of cytochrome *c* peroxidase from *ps. nautica*

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Pseudomonas (Ps.) nautica strain 617 is a halophile marine denitrifying bacterium, that when grown under micro-aerophilic conditions, can express a cytochrome *c* peroxidase (CCP). Like the others CCPs described in *Ps. aeruginosa* and *Pa. denitrificans* [1] the CCP isolated from *Ps. nautica* contains two functionally distinct haem groups, one of which (the electron-transferring haem) acts as reservoir for one of the electrons required to reduce hydrogen peroxide, while the other (the peroxidatic haem) is the active site. The spectroscopic studies showed that the CCP from *Ps. nautica* contains most probably two types of Ca^{2+} binding sites, required for the activation of the enzyme, as described for the CCP from *Pa. denitrificans* [1]. Monohaemic cytochrome c_{552} from *Ps. nautica* was identified as the physiological electron donor, with a half-saturating concentration of 122 μM and allowing a maximal catalytic-centre activity of 116000 min^{-1} [2]. Catalytic currents were observed by direct electrochemistry in the system CCP- c_{552}-hydrogen peroxide.

We thank Fundação para a Ciência e a Tecnologia for financial support (BD/18295/98).

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Nuclease activity of a Mn(III) complex with N-(4,6-dimethyl-2-pyrimidyl)sulfonamide

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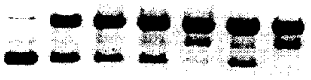
Manganese complexes have been recently reported as efficient chemical nucleases. The study of this systems constitues an interesting field in biomedical sciences.1,2 Herein a new complex of Mn (III) with N-(4,6-dimethyl-2-pyrimidyl)sulfonamide (sulfamethazine) was sinthesized and characterized. The nuclease activity of this complex was determinated by electrophoretic study and corroborated by AFM (Atomic Force Microscopy), a powerful method for observing interactions between DNA and other molecules.

The results indicated that 40 μM of the complex cleavages efficiently DNA. At this concentration supercoiled DNA is transformed into nicked and linear DNA. Currently, futher investigations on the mechanism of DNA breakage by the complex are in study.



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Unusual chloride *vs.* macrocycle competition in Ru([9]aneS3)($HCpz_3$)Cl^+: a combined theoretical and experimental study.

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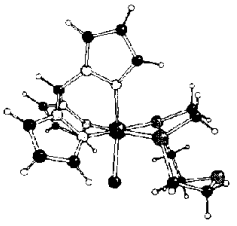
In the solid state, [Ru[9]aneS$_3$)(HCpz$_3$)Cl] Cl ([9]aneS$_3$=1,4,7-trithiacyclononane and HCpz$_3$=tris-pyrazolyl) presents an (S,S',S'',N,N',Cl) coordination mode. While ^{1}H-NMR results seem to indicate a fast and total conversion to a (S,S',S'',N,N',N'') coordination in solution, with the displacement of the Cl^- ion, *ab initio* calculations at the B3LYP level predict a (S,S',N,N'N'',Cl) coordination as the lowest energy minimum, with a bound chloride (See Figure). In this work, this unusual competition between the Cl^- anion and the tridentate macrocycles for Ru^{II} coordination is studied by combined experimental and theoretical methods.



The Portuguese Ministry of Science and Technology is acknowledged for financial support.

The use of high field/ frequency EPR (HF-EPR) in studies of radical and metal sites in proteins and small inorganic models.

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Low temperature EPR spectroscopy with frequencies between 95 GHz and 345 GHz (HF-EPR) and magnetic fields up to 12 Tesla have been used to study radicals and metal sites in proteins and small inorganic model complexes. We have studied radicals, Fe, Cu and Mn containing proteins. For S=1/2 systems, HF-EPR can resolve the g-tensor anisotropy. It was used in mouse ribonucleotide reducase[1] to show the presence of a hydrogen bond to the tyrosyl radical oxygen. At 285 GHz the type 2 copper(II) signal in the complex enzyme laccase[2] is clearly resolved from the Hg(II) containing laccase peroxide adduct[2] (samples from E.I. Solomon's group). Simple metal sites with S>1/2 can be described by the spin Hamiltonian: $\mathcal{H}_S = \mu_B \mathbf{BgS} + D[S_z^2 - S(S+1)/3 + E/D\ (S_x^2 - S_y^2)]$. From HF-EPR, a precise D-value can be determined by: **I)** shifts of the g_{eff} for large D-values and half-integer spin as observed at 345 GHz on an Fe(II)-NO-EDTA complex (S=3/2 with D=11.5 cm^{-1}, E=0.1 cm^{-1} and $g_x=g_y=g_z=2.0$); **II)** obtaining the high-field limit spectrum for intermediate D values as observed in S=5/2 Fe(III) EDTA and transferrin[3]. In binuclear Mn(II) substituted mouse ribonucleotide reductase R2 protein the two weakly interacting Mn(II) at X-band could be observed as decoupled Mn(II) at 285 GHz.

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Supported by the Norwegian Research Council, the *Iron-oxygen Protein Network* (ERBMRFXCT980207) and ERBFMGECT950077 of EU TMR programmes

Photoinduced electron transfer within an artificially created protein–protein complex

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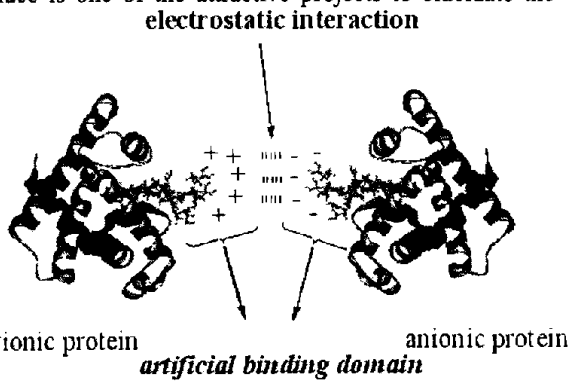
Construction of an artificial binding domain on a protein surface is one of the attractive projects to elucidate the mechanism of biological electron transfer (ET) via non-covalently linked protein–protein interaction. Thus, we have recently prepared unique myoglobins having an artificially created binding domain which can interact with a redox partner such as quinone, methyl viologen, and cytochrome *c*.



For example, zinc myoglobin having a positively charged cluster at the terminal of heme-propionates binds with anthraquinone-2,7-disulfonate with an association constant of 1.5 x 10^5 M^{-1}.[1] Within the complex, photoinduced singlet ET was monitored from the zinc myoglobin to the quinone with a rate constant of 2.0 x 10^9 sec^{-1}. Furthermore, it is found that the myoglobin forms the stable complex with a negatively charged protein. Here, we wish to present ET reactions within the artificial protein–protein complex.

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Catalysis of water-insoluble aldehydes by *D. gigas* Aldehyde Oxidoreductase

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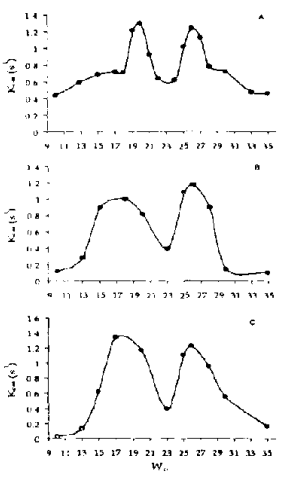
The kinetic behavior of aldehyde oxidoreductase (AOR) isolated from the sulfate reducing bacteria *Desulfovibrio gigas* was studied in reverse micelles of sodium bis (2-ethylhexyl) sulfosuccinate (AOT) in isooctane. AOR is a homodimeric protein that catalyses the conversion of aldehydes to carboxylic acids. The influence of the reverse micelle water content on the enzyme kinetic was investigated and enzymatic activity could be observed either for water-soluble (benzaldehyde-A) as for water-insoluble substrates (octaldehyde–B and decylaldehyde-C). With all tested substrates, a non linear dependence of K_{cat}^{ov} as a function of the micelle size (W_o=H_2O/AOT) was recorded with peaks around W_o = 20 and 26, which can be assigned to different protein conformations - the monomer and the dimer, respectively. The catalytic constant (K_{cat}^{ov}) at these two W_o values is comparable to those obtained in a non-micelle system, using benzaldehyde as substrate. Contrary to these observations in reverse micelles, activity was not detected in the aqueous fractions of octaldehyde and decylaldehyde. Experiments performed with spin labeled reverse micelles elucidated that when solubilized in this system, the water-insoluble aldehydes are located within the surfactant molecules and so, in a favorable position to interact with the solubilized protein molecule. The results clearly show that it is possible to follow the benzaldehyde AOR activity in reverse micelles, extend the range of substrate molecules to water-insoluble ones and observe the enzyme subunit functionality[1].



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Interaction of a water soluble porphyrin with human serum albumin

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Numerous studies dealing with the biological and photophysical properties of porphyrins have been performed over the years.[1,2] Nevertheless, the mechanism and site of action of certain water-soluble porphyrins at cellular level still demand careful attention. Thus, a better understanding of the interaction of porphyrins with cellular targets like membranes, proteins or nucleic acids is essential.

In this communication we report some studies with a transport protein in the blood plasma, human serum albumin (HSA), which binds to a wide variety of substances such as metals, fatty acids, and a large number of therapeutic drugs. The interaction of HSA with *meso*-tetrakis(p-sulfonatophenyl)porphine (TSPP) was followed by changing the protein concentration and the solution pH. Spectroscopic data (CD and fluorescence) put in evidence the important role of HSA on the porphyrin aggregation. The increase on temperature leads to porphyrin de-aggregation while favouring HSA-TSPP binding.

HSA-TSPP interaction was also examined by following the intrinsic fluorescence of the only tryptophan residue (Trp^{214}) of HSA. Fluorescence quenching by acrylamide and TSPP itself gives insight into the specificity of the target site(s) and the nonradiative process induced upon binding.

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DNA interaction with divalent and trivalent metal ions at different temperatures studied by vibrational circular dichroism (VCD) and IR spectroscopy

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DNA interaction with three metal ions, namely Cr^{3+}, Mn^{2+} and Cu^{2+}, was studied by Vibrational Circular Dichroism (VCD) spectroscopy. VCD is a relatively new technique in this field. This method adds circular dichroism ability to conventional vibrational spectroscopy and thus combines the advantages of IR absorption and Electronic CD (ECD) spectroscopy.

Trivalent and higher valence metal ions are able to induce DNA condensation. A drastic increase of intensity of VCD spectra similar to that observed in ECD occurred during DNA condensation induced by Cr^{3+} ions, indicating VCD's ability to detect DNA condensation. In contrast to ECD, the combination of VCD and IR absorption has enabled the observation of DNA condensation and the determination of the secondary structure of the DNA inside condensed particles in the same experiment.

Divalent metal ions are known to induce DNA aggregation at increased temperatures, however some authors have indicated an additional possibility of DNA condensation in these conditions. Our study confirmed that Mn^{2+} ions are indeed capable of inducing DNA condensation at elevated temperatures. Furthermore, it was possible to distinguish between DNA condensation and aggregation, which is hardly feasible with other techniques.

Analysis of VCD spectra of DNA - Cu^{2+} complexes favored base-phosphate chelation and "sandwich" complex models and made it possible to visualize the structure of the complex based on these models.

A search for a cluster in pMMO: the broad g=2.1 signal revisited

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Particulate methane monooxygenase (pMMO) catalyzes the oxidation of methane to methanol using dioxygen. EPR spectra of *M. capsulatus* (Bath) are obtained at 77 K and at 12 K as the concentration of cupric ion added to the growth medium varies from 0 to 20 μM. With Cu in the media, in addition to the type 2 Cu^{2+} signal, a broad signal at g=2.1 is observed as previously reported by others (Nguyen *et al.*, J. Biol. Chem. 269(21) 14995-15005(1994)). Also, a free radical signal and a signal at g=2.01 attributed to an iron-sulfur cluster is observed. If Cu^{2+} is not added to the medium, little type 2 Cu^{2+} EPR signals are obtained, but signals at g=16 and g=1.94, 1.86, and about 1.75 are observed and tentatively assigned to the μ-oxo-bridged binuclear iron cluster in sMMO, in accord with previously reported results (B. G. Fox, K. K. Surerus, E. Münck and J. D. Lipscomb J. Biological Chemistry, 1988, 263, 10553-56).

Two pieces of data support the hypothesis that the broad signal is a pMMO signal. First, the broad g=2.1 signal increases as the Cu^{2+} concentration in the medium increases. The concentration of type 2 Cu^{2+} is about 500 μM and the broad signal is at least 100 μM. pMMO is the only protein that matches these high concentrations. Second, the broad signal, as well as the signals from type 2 Cu^{2+}, is found in the membrane fraction, where pMMO is the dominant protein.

The broad signal is (i) difficult to saturate at 12 K, an indication that it may arise from multiple spins, (ii) sustains for months if whole cells or membranes from (Bath) are stored at 77 K, (iii) disappears over time after treatment of membrane fractions with hypochlorite, suggesting that the site is not fully oxidized, and (iv) retains the g=2.1 value at S-band, suggesting that the signal arises from a ΔM_s=+/-1/2 transition from a site with multiple spins or the signal arises from a mixed valence multinuclear site.

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Solution structure of the unbound, oxidized photosystem I subunit PsaC, containing [4Fe-4S] clusters F_A and F_B. A conformational change occurs upon binding to photosystem I.

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The PsaC subunit of Photosystem I (PS I) is a small (9.3 kDa) protein that harbors binding sites for two [4Fe-4S] clusters, usually referred to as F_A and F_B. PsaC is presumed to evolve from a soluble bacterial ferredoxin and has a high sequence similarity to di-cluster ferredoxins in the vicinity of the iron-sulfur cluster consensus binding sites CxxCxxCxxxCP. PsaC has an additional sequence insertion between the binding sites for the two clusters as well as a C-terminal extension. This work presents the three-dimensional NMR solution structure of recombinant, oxidized, unbound PsaC from *Synechococcus* sp. PCC 7002. Constraints are derived from homo- and heteronuclear, two- and three-dimensional NMR data. Significant differences are outlined between the unbound PsaC structure presented here and the available X-ray structure of bound PsaC as an integral part of the whole cyanobacterial PS I complex[1]. The observed structural features in solution may be relevant for the proposed stepwise assembly of the stromal PsaC, PsaD and PsaE subunits to the PS I core heterodimer.

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Control of peptide conformation by palladium(II) coordination

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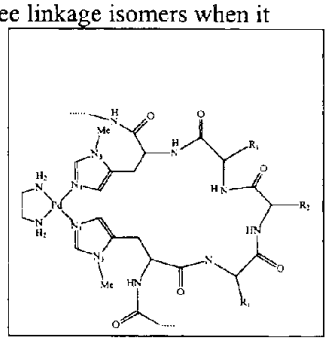
We have previously reported[1] that the pentapeptide $AcHisAlaAlaAlaHisNH_2$ forms three linkage isomers when it binds to $Pd(en)^{2+}$ in dmf, with the metal bound by histidine imidazole N_1,N_1, N_1,N_3, and N_3,N_1. For the most favoured isomer (N_1,N_1), the structure obtained in solution by NMR methods showed that the peptide conformation corresponded to a single turn of an α-helix. We have subsequently shown that there is similar linkage isomerism in aqueous solution, and that an α-helical conformation is imposed on the pentapeptide in the major isomer. We have synthesized a number of peptides with amino acid sequences corresponding to those near the active site of the zinc-containing enzyme thermolysin. To avoid complications from linkage isomerism, the imidazole N_3 atoms have been methylated, so that only the N_1 atoms bind to the palladium (Figure). Histidine residues so modified are indicated as His*. For the decapeptide $AcHis^*GluLeuThrHis^*AlaValThrAspTyrNH_2$ in dmf, $Pd(en)^{2+}$ is bound by the two imidazole N_1 atoms, and the solution structure determined by NMR shows that a helical conformation is imposed on the whole peptide, with some "fraying" at the C-terminal end. Similarly for a peptide containing 15 amino acid residues, with the two substituted histidine residues at the center, an α-helical conformation is imposed on the whole peptide in dmf. In water, for the 10- and 15-amino acid peptides, there is no evidence for helical structures away from the immediate vicinity of the palladium binding site.



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Nickel thiophenolate complexes: can a stable bound thiyl radical be formed?

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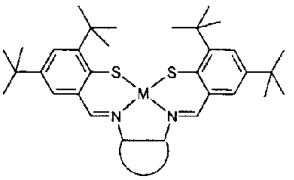
Interest in the biochemical exploitation of protein radicals has been increasing dramatically over the last 10 years as new enzymatic systems are characterised[1]. Enzymes that incorporate amino acid or modified amino acid based radicals include ribonucleotide reductases, cytochrome *c* peroxidase, copper-dependent amine oxidases and galactose oxidase. A thiyl radical species (cysteine) has been shown to be involved in the catalytic cycle of ribonucleoside triphosphate reductase.



Nickel complexes of thiolate ligands have been of interest in modelling hydrogenase enzymes and examining the effect of sulfinate and sulfenate formation[2]. In an attempt to characterize stabilized metal bound radical species a series of small molecule complexes have been synthesized and characterized. Similar strategies to stabilize metal radical species to those successfully exploited in synthesizing small molecule galactose oxidase mimics, containing metal bound phenoxy radicals, have been adopted[3].

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Characterization of diiron-peroxo complexes with asymmetric polypyridine ligands

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The proteins, such as hemerythrin (Hr), methane monooxygenase (MMO), and ribonucleotide reductase (RNR), function as a dinuclear iron center. Hr, which exists in several marine invertebrate phyla, plays an important role for carrying dioxygen molecule to the cell. The X-ray structure of deoxy Hr revealed that the diiron active site formed asymmetric core comprised of six- and five-coordinate iron atoms, in which the former acts as an electron poor and the latter plays as a binding site of dioxygen. The oxygen molecule binds reversibly to the coordinatively unsaturated iron site as a hydroperoxo species in an end-on fashion (Figure 1). We prepared newly designed diiron complexes, $[Fe_2(bppdo)(CH_3COO)](ClO_4)_2$ (**1**) (bppdo = *N,N*-bis(6-pivalamido-2-pyridylmethyl)-*N',N'*-bis(2-pyridylmethyl)-1,3-diaminopropan-2-ol), that have two iron centers with different coordination geometries as a structure/function model of Hr.

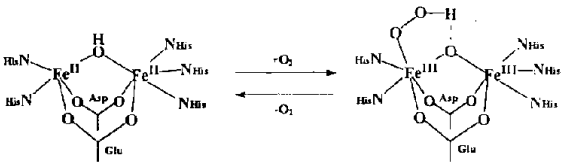

Figure 1. Mechanism for oxygenation of Hr

The crystal structure of **1** revealed that the two iron atoms were bridged by the acetate ion and the alkoxo group of bppdo, and that one iron atom formed seven-coordinate capped octahedron and another one had six-coordinate octahedron with a dioxygen-accessible vacant site. Exposure of dioxygen to the acetone solution of **1** at −78 °C afforded a reddish purple speceies (**2**). The UV-vis spectrum of **2** exhibited an intense band at 537 nm (e = 1800 $M^{-1}cm^{-1}$) attributable to a LMCT band of peroxo to Fe(III) ion, indicating the formation of peroxo adduct. ESR spectrum of **2** was silent due to a strong antiferromagnetic coupling of the two Fe(III) centers.

Structure-activity relationship of antitumour ruthenium azole complexes

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Antitumour and antimetastatic activity was documented for a number of ruthenium(II) and ruthenium(III) complexes.[1,2] One of them, NAMI-A, Him[$RuCl_4$(DMSO)im], where DMSO = dimethylsulphoxide and Im = imidazole, reached clinical trials in 1999. Ruthenium(III) complexes are supposed to be prodrugs, because of their inertness under physiological conditions. They are able, however, to undergo a reduction in the hypoxic environment of the tumour leading to ruthenium(II) species. These are more labile and interact easier with the DNA, which might be the target for antitumour ruthenium agents.

We report herein on the synthesis of ruthenium(III) chloride complexes, containing a different number of indazole ligands (Fig. 1), their antitumour activity and redox properties.

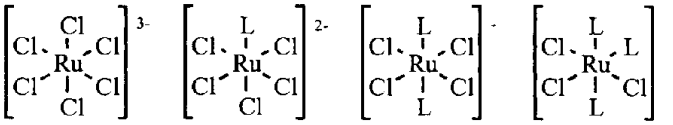

Fig. 1. $RuCl_{6-n}L_n$ complexes, with n = 0 - 3 and L = indazole.

Increasing the number of indazole ligands from 0 to 3 facilitates the reduction of ruthenium(III) to ruthenium(II). A relationship between the cytotoxicity and the number of coordinated azole ligands has also been found.

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2. Clarke M.J., Zhu, F., Frasca, D.R. Chem. Rev. 99, 2511-2533 (1999).

We gratefully acknowledge the FWF (Austrian Science Fund) for financing the project P14087.

Capillary electrophoresis study of the interaction between antitumour-active ruthenium(III) azole complexes and nucleoside bases

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Ruthenium(III) complexes of the general formulae HL[$RuCl_4L_2$], with two *trans*-located azole ligands, show remarkable antitumour activity.[1-3] Although the mechanism of their action has not definitely been understood yet, it is generally believed that DNA might be the target for antitumour-active ruthenium species.

Herein we report on the study of the interaction of Him *trans*-[$RuCl_4(im)_2$] and Hind *trans*-[$RuCl_4(ind)_2$] with four nucleoside bases GMP, AMP, CMP and TMP by means of capillary electrophoresis.

We found that the complexes studied bound preferentially to GMP and AMP. Under competitive conditions no binding to CMP occurred. TMP was able to coordinate to ruthenium azole species only being deprotonated, what is excluded under physiological conditions. In addition, it was also established that the amount of bound nucleoside base is pH dependent, increasing at lower pH. This seems to be of biological relevance, taking into account the lower pH values in solid tumours.

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We gratefully acknowledge the FWF (Austrian Science Fund) for financing the project P14087.

Mimicking the structure and function of the photosystem II water oxidase active site tetramanganese complex using synthetic complexes

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In oxygenic photosynthetic organisms water oxidation is catalyzed by a tetranuclear manganese complex in Photosystem II (PSII Mn_4). The structures of this metal cluster complex at each stage in the catalytic cycle are not well established. Furthermore the mechanism of action is not well understood. We are using synthetic model compounds to address questions about the PSII Mn_4 structure and function. In this presentation one area of emphasis will be the dramatic structural changes exhibited by the 'dimer-of-dimers' species $[Mn_4O_4(tphpn)_2]^{4+}$ [1] upon oxidation or reduction. Oxidation causes rapid conversion to an adamantane-shaped isomer. Reduction provides, after purification, a species whose EPR spectrum shows remarkable resemblance to that of the PSII S_0 state. The reduced species undergoes a structural rearrangement as well. Similar cluster structural changes may play an important role in biological oxygen evolution catalysis.

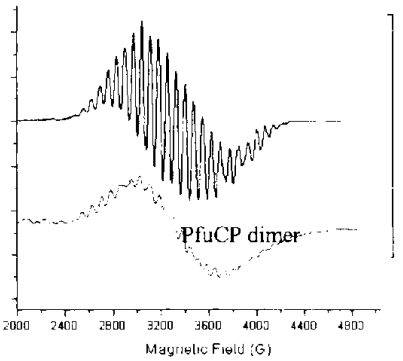

Figure at right: X-band EPR spectra of the PSII S_0 spectral model complex $[Mn_4O_4(tphpn)_2]^{3+}$ (top) and the Photosystem II S_0 state (bottom).

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X-ray crystal structure of *pyrococcus furiosus* carboxypeptidase at 2.2 Å resolution

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The x-ray crystal structure of a novel class of carboxypeptidase (PfuCP) from the hyperthermophilic archaeon, *Pyrococcus furiosus*, has been determined to 2.2 Å resolution. PfuCP is a cobalt-dependent metalloprotease that exists as a homodimer of 59 kDa subunits.[1] The structure of apo-PfuCP was solved by multiple anomalous dispersion (MAD) techniques from a single crystal soaked with ytterbium. A second structure with a lead ion bound at the protein active site was solved by molecular replacement. PfuCP is almost entirely helical with its most predominant feature being a deep channel that transverses the entire length of the protein. Previous sequence analyses had identified a HExxH motif characteristic of metalloproteases. The lead-bound structure confirms the role of this motif in forming the metal-binding site. Two histidines from the HExxH motif and a glutamate from an adjacent helix coordinate the metal atom in a manner that is characteristic of the MA clan of metalloproteases (e.g. thermolysin).[2] Interestingly, the PfuCP structure reveals unexpected homology to the rat neurolysin endopeptidase that catalyzes the zinc-dependent hydrolysis of neuropeptides.[3] By comparison with other metalloproteases in complex with transition-state analogs, substrate was modeled to the active site and a mechanism proposed for the C-terminal peptide bond hydrolysis.

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