Scientific report

Implementation of project PN-II-ID-PCE-2012-4-0488

Title:

REDOX ACTIVATION OF SMALL MOLECULES BIOLOGICALLY RELEVANT METAL CENTERS

The aim of the project has been to investigate the reductive and oxidative activation of small molecules at centers including iron enzymes, cobalamin, and related model systems. Substrates for such experiments were to be hydrogen sulfide (recently acknowledged as important signalling molecule acting on metalloenzymes), as well as sulfur oxides and oxyanions. Enzymes working on halide and carbon oxyanions will offer interesting parallels with the sulfur systems; (mechanisms in, e.g., sulfite reductase or chlorite dismutase still await detailed exploration). Proton reduction is also expected to be implicit in the case of the lower-oxidation states - especially in cobalamin-related models. Computational as well as experimental methods (rapid kinetics, low-temperature spectroscopy) were to be employed to this end. In terms of deliverables, a total of 6 publications with a cumulated impact factor (ISI) of at least 15 points were set as minimal targets.

The number of published works and their cumulated impact factor fall into predictions (exceeding by five times the numbers anticipated, with other contributions still in work):

A.A. Attia, A. Lupan, R.B. King, Polyhedron. 2015, 85, 933-940; doi: 10.1016/j.poly.2014.10.005
A.M.V. Brânzanic, A. Lupan, R.B. King, Organometallics, 2014, 33, 6443-6451; doi: 10.1021/om500801e
A. Lupan, R.B. King, Polyhedron, 2014, 71, 133-141; doi: 10.1016/j.poly.2014.01.010
Dereven’kov, Ilia A.; Salnikov, Denis S.; Silaghi-Dumitrescu, Radu; Makarov, Sergei V.; Koifman, Oscar I. Coordination Chemistry Reviews, DOI 10.1016/j.ccr.2015.11.001
Kakes, Melinda; Cioloboc, Daniela; Tomsa, Adrian-Raul; Silaghi-Dumitrescu, Radu; Damian Grigore. Revue Roumaine de Chimie, 2015, 60(7-8), 707-720
Silaghi-Dumitrescu, Radu; Scurtu, Florina; Mason, Maria; Svistunenko, Dimitri A.; Wilson, Michael T.; Cooper, Chris E. Inorganica Chimica Acta, 2015, 436, 179–183
Attia, Amr A. A.; Silaghi-Dumitrescu, Radu. Journal of Molecular Modeling, 2015, 21, 130-142
Lakk-Bogáth, Dóra; Speier, Gábor; Surducan, Mihai; Silaghi-Dumitrescu, Radu; Simaan, A. Jalila; Faure, Bruno; Kaizer, József. RSC Advances, 2015, 5, 2075–2079
Silaghi-Dumitrescu, Radu; Carrascoza Mayen, Juan Francisco. Studia Universitatis Babeș-Bolyai Seria Chimia 2014, LIX(3), 95-101
A.A.A. Attia, A. Lupan, R. B. King, J. Organomet. Chem. 2015, in press, 10.1016/j.jorganchem.2015.04.010
Attia, Amr A. A.; Silaghi-Dumitrescu, Radu. Journal of Molecular Graphics and Modelling, 2016, 69, 103-110
Szávuly, Miklós István; Surducan, Mihai; Nagy, Emőke; Surányi, Mátyás; Speier, Gábor; Silaghi-Dumitrescu, Radu; Kaizer, József. Dalton Transactions, 2016, 45, 14709-14718

Manuscripts in work include:
R. Silaghi-Dumitrescu, A. Attia et al. Peroxo-Transition Metal Systems: Examples of Electromerism in Palladium Structures
A. Attia, R. Silaghi-Dumitrescu. A quantum chemical study on the reaction mechanism of nickel cysteine dioxygenase.
F. Carrascoza, M. Surducan, A. Kun, R. Silaghi-Dumitrescu – Bleomycin reaction mechanisms
C. Bischin, L. Gaina, R. Septelean, A.C. Mot, R. Silaghi-Dumitrescu et al – TBA
A.A. Attia, D. Cioloboc, R. Silaghi-Dumitrescu – Chlorite dismutase – a hemoprotein with a catalytic mechanism focused on higher-spin states
A. Branzanic, U. Ryde, R. Silaghi-Dumitrescu - High-valent heme-sulfide adducts in sulfite reductase
A. Branzanic, U. Ryde, R. Silaghi-Dumitrescu – Iron-sulfur cluster effects on linkage and redox isomerism in sulfite reductase reactive intermediates
A. Branzanic, U. Ryde, R. Silaghi-Dumitrescu – Accurate computational description of the resting state in sulfite reductase: importance of the heme / iron-sulfur cluster coupling
A. Attia, R. Silaghi-Dumitrescu – Nitrogen activation at low-valent metal centers: what makes a good nitrogenase
M. Surducan, R. Silaghi-Dumitrescu – Linkage and isomerism in heme-SO adducts

To these one may also add contributions to international conferences such as EUROBIC (Zurich, Budapest), ICBIC (Beijing), EUCHEMS, O2BiP (Sheffield, Hamburg), DCIRM -Debrecen, WATOC, as well as national annual conferences in chemistry, biochemistry, molecular modeling, and others.
All team members participated in manuscripts in progress or published. In addition to these, a number of students, graduate or undergraduate, were involved in the experiments, some being present in the lists of authors listed above (ie., Hathazi, Vaida, Stanciu, Mahut, and others). Overall, the publications supported by this project have amassed a total of 70 impact factor points, including one in the Coordination Chemistry Reviews. Besides these contributions, the continued support for the PI’s research group has also made possible a number of publications that also total several tens of impact factor points on related themes (redox activity of bioinorganic centers). One may therefore argue that the funds spent so far have led to a reasonable outcome, especially when one considers the relatively unpredictable work environment in this part of Europe. Issues not covered in the manuscripts listed above, are reviewed in the following paragraphs.

Characterization of sulfide complexes.

After the addition of 16 micromolar sulphide concentration to a concentration of 6 μM MetHb major changes were observed. Stopped-flow UV-vis and EPR experiments ensued. A further proof that the observed compound is a ferric sulfide adduct may come from the fact that this a band indeed characteristic, shown in Figure 1, is formed upon addition of peroxide and sulfur, in this order, to hemoglobin.

![Figure 1. UV-vis spectrum of the reaction MetHb (6 μM) with H2O2 (160 μM) and Na2S (16 μM), phosphate buffer pH 7.4](image-url)
Reactions were also studied with methemoglobin in the presence of liposomes, sulfite and sulphide respectively, in order to see if they produce changes in lipid oxidation. Figure 2 shows that at a wavelength characteristic of the oxidation of lipids (240 nm), the kinetics of the control sample containing only liposome MetHb are different compared with the samples in which the sulfite and sulphide added. The results indicate that there is a gap between the lipid oxidation induction time (a different lag time), but there are differences in the amount of oxidized lipid.

![Graph showing lipid oxidation](image)

**Figure 2.** Lipid oxidation by MetHb (6 µM) in the presence of sulfite (500 µM) and sulfide (16 µM) 240 nm (wavelength specific to the oxidation of lipids), 500 min, pH 7.4 phosphate buffer.

The reaction of the oxy form of globins with chlorite seems to lead to a new class of ligand oxyanion of chlorine. Similar processes have been observed by us and the reactions of hypochlorite, and will be detailed.

Figure 3 summarizes the catalytic cycle of chlorite dismutase obtained from DFT calculations.
Figure 3. Evolution of chlorite dosmutazei catalytic cycle, deduced so far from our calculations; vertical axis is in kcal / mol.

Figure 4 illustrates some of the efforts to explore the reactivity of other halogens - namely, the TPO enzyme catalytic mechanism, responsible, through mechanisms still under discussion, the halogenation (iodination).
Figure 4. DFT data on the activation link in a model hemin OI active site of thyroid peroxidase (Fe (III) with imidazole coordinated trans to IO) as a first step in the set the reactions that lead to physiologically relevant halogenation.

**Hemoprotein reaction with sulfite (SO$_3^-$)**

Possible interactions between hemoglobin / myoglobin and sulfite were studied at three pHs (pH 5, 7 and 11), for the three types of proteins: met, oxy or deoxy. In none of the cases, there was a change in the UV-vis spectrum in time, suggesting that sulfite access to the iron hemin is not possible for these two proteins, or that the potential adduct has spectroscopic signature indistinguishable from the one of the starting materials.

![UV-vis spectra](image)

Figure 5. UV-vis spectra of the met-hemoglobin (A) and deoxy-hemoglobin (B) collected after mixing with 200 mM sodium sulfite.

Sulfite accessibility to heme and hemin has been studied for another heme protein, horseradish peroxidase. As can be seen from Fig. 6, this case did not, yet again, reveal the presence of any intermediate or reaction product spectrum clearly individualized. Data from the literature suggest that with ESR spectra (electron spin resonance) such an interaction could be, instead, detectable, and that this track remains to be further investigated.
Figure 6. UV-vis spectrum of horseradish peroxidase by treating it with 500 mM sulfite, pH 5.

**Reaction of hemoproteins fluoride**

Unlike sulfite, the fluoride anion forms a met-hemoglobin adduct, which is observable in Figure 7. Yet, the spectra of the oxy form and the deoxy remain unaffected in the presence of excess fluoride. Redox experiments on this system are currently underway.

Figure 7. The UV-vis spectra of the met-hemoglobin collected by mixing with 100 mM fluoride.

The fluoride adduct of met-hemoglobin has been oxidized with hydrogen peroxide and ammonium peroxodisulfate or reduced with DOTU to highlight the occurrence of possible intermediates. In Fig. 8A one may observe that by treating the adduct with oxidants ferryl is formed, whereas Fe(II)-CO is observed with DOTU.
Figure 8. UV-vis spectra of met-hemoglobin after mixing it with 100 mM fluoride and: A) 1 mM peroxide and B) 30 mM DOTU.

**Hemoprotein reaction with hypochlorite (HOCl)**

As in the case of fluoride, treatment of met hemoglobin with hypochlorite results in the formation of an adduct, presumably, in this case, Hb-Fe (III) -ClO.

Figure 9. UV-vis spectra of hemoglobin after mixing it with 50 μM HClO, pH 7.

To highlight and characterize the intermediates formed in this reaction, the stopped-flow technique was used. The spectra obtained in the first 2 ms after mixing of the reactants can vantage a series of three consecutive reactions, A> B, B> C and C> D.
Figure 10. A) UV-vis spectra of hemoglobin treated with 466 μM HClO, pH 7, collected within 2 ms after mixing the reactants; B) The four species specific program proposed, based on experimental data fitting of the model A> B, B> C and C> D; C) experimental data fitting model A> B, B> C and C> D.

In the following, are described theoretical investigations (DFT, but sometimes they were supplemented with CASSCF results and QM / MM, where the electronic structure was a complex subject such as Fe-XO adducts, or where the role of matrix protein wanted to be investigated in more detail). Many of these results are in connection with experiments already performed, or at least planned, in our laboratories.
Models of superoxide reductase

Figure 11. The potential energy surface for the Fe-O bond breaking in the SOR-dioxygen. Dissociation is only possible for high-spin state (S = 2).

Figure 12. The potential energy surface for the Fe-O bond breaking in the SOR-dioxygen. (imidazole-axial). Again the only possible process model is where high spin (S = 2).

Models sulfite reductase / ferryl interaction with sulfide (Fe-OS models)

Models contain Fe (III) porphyrin bound to axial ligands described below, located trans to either methylthiolate (SMe) or imidazole (I)
Figure 13. Chart of potential energy versus OS distance for different models (state of spin S = 3/2 and 1/2). Point 0 kcal / mol is arbitrarily taken as the starting geometry steady state spin S = 1/2 for the given pattern. For model SMe-Fe-OSH2 the final point on the graph corresponds to an OS distance of 2.54.

Additional calculations were performed on similar models of type Fe-OOH studying OO bond elongation with direct relevance to the oxygen-oxygen bond breaking and heme oxygenase the mechanism. Another set of calculations was made along the reaction coordinate of bond breakage for OS Fe-OS models (with the same type axial ligands and Imi and SMe).
 Models of chlorite dismutase

Figure 15. Potential energy surface for one of the isomers, breaking Fe-O and Cl-O bonds. The results show that Cl-O bond breaking is feasible only in the high spin model in terms of energy.

Figure 16. Potential energy surface for one of the OOClO isomers. The results show that none of the processes is possible.
Fe-peroxynitrite models (interaction of peroxonitrate with Fe (II) and Fe (III) in molecules of biological interest)

Figure 17. The potential energy surface for the formation / cleavage of the OO(NO2) or OO-NO2 bond in the peroxonitrate ferric adduct. These are showing both processes as possible, with preference for the dissociation along the oxygen-oxygen bond.

Figure 18. The potential energy surface for the formation / cleavage of the OO (NO2) bond in the case of ferrous adduct (S = 1). The results show an exothermic process that runs without any barrier.
Oxides and oxyanions, XOₙ: computational characterization

Figure 19 illustrates a set of models investigated using DFT, in terms of their electronic and geometric structure, as well as in terms of their reactivity (linkage isomerism, activation of oxygen-heteroelement bond). A complete set of results is available, and will be presented in ensuing manuscripts.

Figure 19. Non-heme models employed in order to explore sulfur oxide/oxyanion binding; M06L/6-31G**.

Figure 20 illustrates some of the examined potential energy curves, towards bond formation/breaking in the models illustrated in Figure 19. By far, HSO₃⁻ appears the most facile target.
Figura 20. Potential energy curves for liberating activated oxygen atoms, in compounds illustrated in Figure 19.

**Siroheme models**

The active site of sulfite reductase is involved in a mechanism so far shaped out as in Figure 21, as proposed by others based on their crystallographic studies, and detailed by our previous DFT data on simplified models. Figure 22 and Table 1 illustrate some of our current efforts to expand beyond those simple models, and explore the role of the siroheme modification vs. a regular $b$-heme, and the role of the iron-sulfur cluster bridged to the heme by a cysteine.
Figure 21. Comparative models of the heme, employed in our current studies.

Figura 22. Comparative models of equatorial and trans ligation, employed in the current studies.
<table>
<thead>
<tr>
<th>Species</th>
<th>Spin</th>
<th>Energy (a.u)</th>
<th>ΔE (kcal/mol)</th>
<th>Energy (a.u)</th>
<th>ΔE (kcal/mol)</th>
<th>Energy (a.u)</th>
<th>ΔE (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1/2</td>
<td>-3316.7924</td>
<td>0.00</td>
<td>-6410.0774</td>
<td>0.00</td>
<td>-11278.2182</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>-3316.7596</td>
<td>20.64</td>
<td>-6410.0633</td>
<td>8.82</td>
<td>-11278.2066</td>
<td>7.29</td>
</tr>
<tr>
<td></td>
<td>5/2</td>
<td>-3316.7572</td>
<td>22.13</td>
<td>-6410.0569</td>
<td>12.87</td>
<td>-11278.0883</td>
<td>81.56</td>
</tr>
<tr>
<td>3</td>
<td>1/2</td>
<td>-3240.4287</td>
<td>0.00</td>
<td>-6333.7241</td>
<td>0.00</td>
<td>-11201.8623</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>-3240.4157</td>
<td>8.21</td>
<td>-6333.7108</td>
<td>8.34</td>
<td>-11201.8492</td>
<td>8.20</td>
</tr>
<tr>
<td></td>
<td>5/2</td>
<td>-3240.4017</td>
<td>16.99</td>
<td>-6333.5778</td>
<td>91.80</td>
<td>-11201.8492</td>
<td>91.80</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>-3240.5085</td>
<td>0.00</td>
<td>-6333.7194</td>
<td>0.00</td>
<td>-11202.5624</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-3240.5008</td>
<td>4.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>-3241.0125</td>
<td>2.56</td>
<td>-6334.3093</td>
<td>0.00</td>
<td>-11202.4539</td>
<td>562.99</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-3241.0165</td>
<td>0.00</td>
<td>-6334.3071</td>
<td>1.42</td>
<td>-11203.5111</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-3240.9870</td>
<td>18.57</td>
<td>-6334.2897</td>
<td>12.32</td>
<td>-11203.5111</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>-3241.3941</td>
<td>0.00</td>
<td>-6334.7964</td>
<td>1.86</td>
<td>-11202.8981</td>
<td>25.68</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-3241.3567</td>
<td>27.31</td>
<td>-6334.7993</td>
<td>0.00</td>
<td>-11202.9390</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-3241.3737</td>
<td>12.82</td>
<td>-6334.7823</td>
<td>10.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-3165.0191</td>
<td>3.93</td>
<td>-6258.4223</td>
<td>15.24</td>
<td>-11126.5371</td>
<td>17.21</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-3165.0254</td>
<td>0.00</td>
<td>-6258.4466</td>
<td>0.00</td>
<td>-11126.5646</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-3164.9770</td>
<td>18.57</td>
<td>-6258.4389</td>
<td>4.81</td>
<td>-11126.5567</td>
<td>4.94</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>-3165.2455</td>
<td>3.93</td>
<td>-6258.5488</td>
<td>0.00</td>
<td>-11126.6916</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>-3165.2132</td>
<td>20.22</td>
<td>-6258.4883</td>
<td>37.97</td>
<td>-11126.6766</td>
<td>9.36</td>
</tr>
<tr>
<td></td>
<td>5/2</td>
<td>-3165.3282</td>
<td>0.00</td>
<td>-6258.5398</td>
<td>0.00</td>
<td>-11126.5343</td>
<td>0.00</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>-3165.3264</td>
<td>1.09</td>
<td>-6258.5126</td>
<td>17.06</td>
<td>-11126.6900</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-3165.2955</td>
<td>20.48</td>
<td>-6258.5110</td>
<td>18.08</td>
<td>-11126.6753</td>
<td>10.23</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>-3165.3409</td>
<td>0.00</td>
<td>-6258.4063</td>
<td>0.00</td>
<td>-11126.5867</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>-3165.3103</td>
<td>19.18</td>
<td>-6258.3761</td>
<td>18.90</td>
<td>-11126.5729</td>
<td>8.63</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>-3165.2437</td>
<td>60.97</td>
<td>-6258.3645</td>
<td>26.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>-3165.9361</td>
<td>0.00</td>
<td>-6259.1496</td>
<td>0.00</td>
<td>-11127.2944</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>1/2</td>
<td>-3165.9042</td>
<td>20.01</td>
<td>-6259.0697</td>
<td>50.09</td>
<td>-11127.2867</td>
<td>8.93</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>-3165.8892</td>
<td>29.40</td>
<td>-6259.1074</td>
<td>26.44</td>
<td>-11127.3009</td>
<td>0.00</td>
</tr>
<tr>
<td>12</td>
<td>1/2</td>
<td>-3090.0781</td>
<td>0.00</td>
<td>-6183.3849</td>
<td>0.31</td>
<td>-11051.5242</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/2</td>
<td>-3090.0757</td>
<td>1.50</td>
<td>-6183.3854</td>
<td>0.00</td>
<td>-11051.5244</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>5/2</td>
<td>-3090.0446</td>
<td>21.06</td>
<td>-6183.3639</td>
<td>13.48</td>
<td>-11051.5152</td>
<td>5.77</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>-3090.1398</td>
<td>16.07</td>
<td>-6183.3499</td>
<td>0.00</td>
<td>-11051.4520</td>
<td>58.08</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-3090.1654</td>
<td>0.00</td>
<td>-6183.3774</td>
<td>4.67</td>
<td>-11051.5446</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>-3090.1387</td>
<td>16.77</td>
<td>-6183.3551</td>
<td>18.68</td>
<td>-11051.5103</td>
<td>21.53</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>-3090.6968</td>
<td>6.25</td>
<td>-6183.9961</td>
<td>0.00</td>
<td>-11052.0858</td>
<td>35.67</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>-3090.7067</td>
<td>0.00</td>
<td>-6183.9375</td>
<td>36.79</td>
<td>-11052.1426</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>-3090.6713</td>
<td>22.23</td>
<td>-6183.9716</td>
<td>15.42</td>
<td>-11052.1388</td>
<td>2.43</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1/2</td>
<td>-3090.7001</td>
<td>33.79</td>
<td>-6183.8983</td>
<td>0.00</td>
<td>-11052.1418</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>-3090.7540</td>
<td>0.00</td>
<td>-6183.8682</td>
<td>18.84</td>
<td>-11052.1408</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>5/2</td>
<td>-3090.7430</td>
<td>6.86</td>
<td>-6183.8612</td>
<td>23.26</td>
<td>-11052.1277</td>
<td>8.87</td>
</tr>
<tr>
<td>16</td>
<td>1/2</td>
<td>-3091.3102</td>
<td>0.00</td>
<td>-6184.5941</td>
<td>0.00</td>
<td>-11052.7279</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>3/2</td>
<td>-3091.2875</td>
<td>14.22</td>
<td>-6184.5812</td>
<td>8.05</td>
<td>-11052.7135</td>
<td>9.01</td>
</tr>
<tr>
<td></td>
<td>5/2</td>
<td>-3091.2792</td>
<td>19.41</td>
<td>-6184.5751</td>
<td>11.93</td>
<td>-11052.7119</td>
<td>10.05</td>
</tr>
</tbody>
</table>

**Oxides and oxyanions of chlorine and sulfur: experimental characterization**

Figure 23 presents EPR on myoglobin treated with chlorite, frozen at 30 s after mixing. Together with UV-vis spectra, these data support a mechanism whereby chlorite coordinates to iron directly, then yielding an EPR-silent state able to oxidize nearby amino acids – hence most probably ferryl.
Figure 23. Spectre RES colectate pentru myoglobin at 30 de seconds after mixing with chlorite, la pH 7 (A) and 10 (B).

Figure 29 illustrates spectra which may be interpreted to be due to a Fe(IV)-sulfido adduct in a heme protein. Figure 30 confirms the oxidized state of this species.
Figure 29. A. SulfoferrylHbFe(IV)=S – formation from metHbFe(III)-SH (7 µM, in reaction with an of iridate at pH 9.5. B. Control experiment without sulfide, leading to classical (oxo) ferryl. Species A, when treated with sulfide, gives rise to the green spectrum which symptomatic of sulfheme and thus indicates the prior presence of a high-valent form (since sulf-heme is known to only occur via high-valent species). C. metHbFe(III)-SH dissociation at pH 5-9 is much slower than direct oxidation by iridate, suggesting that the oxidation occurs directly on the ferric-sulfide adduct.
Figure 30. EPR spectra collected upon oxidation of metHbFe(III)-SH with iridate, leading to an EPR-silent species – most likely Fe(IV)-sulfido. One may also note no significant increases in the isotropic features specific to heme degradation – suggesting a generally clean redox process at the heme.

Figures 31 and 32 illustrate UV-vis and EPR spectra representative for experiments where synthetic macrocycles (model compounds) react with various oxidants of interest. Generally, these indicate that in some cases new adducts have indeed been formed, as expected.

Figure 31. UV-vis and EPR spectra collected during the reaction of ferric tetrasulfophthalocyanine with ligands and redox partners: chlorite, peroxide, sulfite, iridate, hypochlorite.
Figure 32. UV-vis and EPR spectra collected during the reaction of Co(III) tetrathiosulfophthalocyanine with ligands and redox partners: chlorite, peroxide, sulfite, iridate, hypochlorite.

Kinetic and spectroscopic evidence for complexes of sulfur oxyanions with cobalamin were also collected in UV-vis and EPR experiments, as detailed in manuscripts currently in work. Among the targets has been a cobalamin-dithionite adducts, whose main features are illustrated in Figure 33 cf. DFT calculations; one may note the intact Co-S bond and the delocalization of the spin density in two different manners in the respective base-on and base-off versions.

Figure 33. M11/6-31g(d,p) data on cobalamin-dithionite adducts (S=1/2, anionic).
Similar observations were collected on a series of other protein and model systems; such targets have included myoglobin, plant hemoglobins, vertebrate hemoglobins, catalase, synthetic porphyrins complexed with various metals (Fe, Cu, Cu, Zn), cobalamin, cobinamide, phthalocyanine and others.

Project manager,

Radu Silaghi-Dumitrescu