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Redox-Induced Binding of $[(tacn)Re^{II}Br(CO)_2]^+$ to Guanine, Oligonucleotides, and Peptides**

Fabio Zobi* and Roger Alberto[a]

Drug discovery and development based on purely organic molecules is a main focus of pharmaceutical research in industries and at universities. Motivated by the impact of cisplatin and its successors, metal-based drug research persists as an incentive and an innovative field with many facets.[1–3] A growing number of comprehensive reviews underscores the vivid research carried out with metals in medicine.[4–8] When designing metal-based drugs, not only structural aspects have to be taken into account. Whereas pharmaceuticals often follow the key–lock principle, metals introduce the feature of metal-centered reactivity, as found in for example, cisplatin with the irreversible alkylation of DNA.[9,10] Many of the currently investigated complexes follow this concept. Examples are the prominent $[Ru^{II}(arene)]^{2+}$ fragment[3] and Ru^{III} complexes of the $[Ru^{III}Cl_4(imz)(dmso)]^-$ type (imz = imidazole derivative). NAMI-A, a Ru^{III} complex, has entered Phase II clinical trials.[11] With the exception of NAMI-A, bioactive metal complexes are frequently low spin d^6 and "alkylate" biologically relevant sites. It has been proposed that NAMI-A is reduced from $Ru^{III} \rightarrow Ru^{II}$ in vivo, thus, acting as a kind of "prodrug".[12–14] We present in this study a well behaved and characterized Re^{II}/Re^{I} d^5/d^6 system for which redox activity is induced upon target binding, thereby causing irreversible metalation of amino acids, peptides or nucleobases such as 9-methylguanine (9Me-G) or guanosine monophosphate (GMP). The scope of the presented Re^{II}/Re^{I} redox couple is a facile and site specific introduction of a robust rhenium complex in small or large biomolecules.

Complexes of the type $[(L^3)Re^{I}(CO)_3]$ are only oxidized at high potentials since the 3 CO groups stabilize Re^{I} efficiently through π-back donation. $[(Cp)Re^{I}(CO)_3]$ (**1**) for instance was oxidized to $[(Cp)Re^{II}(CO)_3]^+$ (**2**) at +1.30 V.[15] The potentials change substantially if one CO is replaced by a donor ligand. As recently described,[16] $[Re^{I}Br(tacn)(CO)_2]$ (**3**; TACN = 1,4,7-triazacyclononane) showed a fully reversible one electron oxidation at moderate $E_{1/2} = +0.20$ V versus Ag/AgCl whereas $[Re^{I}(tacn)(CO)_3]^+$ could not be oxidized up to +1.5 V. This different electrochemical behavior mirrors the distinctly different electronic properties of rhenium after replacing CO for the σ-donor Br^-.[16] Accordingly, **3** could be chemically oxidized in CH_2Cl_2 with ferrocenium (Fc^+, $E_{1/2} = 0.56$ V) to yield the yellow Re^{II} complex $[Re^{II}Br(tacn)(CO)_2]PF_6$ (**4**[PF_6]) in quantitative yield. The authenticity of **4** was confirmed by an X-ray structure analysis. An ORTEP together with important bond lengths and angles is given in Figure 1.

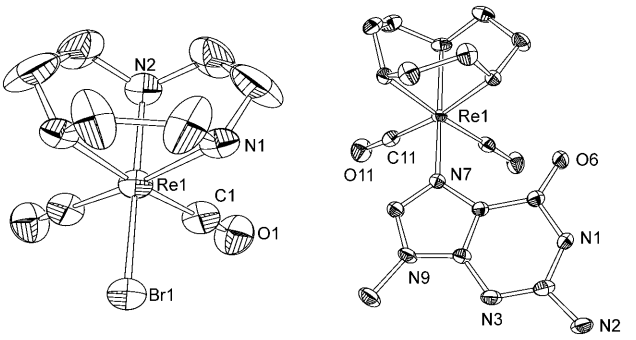

Figure 1. ORTEP presentation of $[ReBr(tacn)(CO)_2]^+$ (**4**, left) and $[Re(tacn)(9Me\text{-}G)(CO)_2]^+$ (**6**, right) . Important bond lengths [Å] and angles [°] for **4**: Re1–C1 1.939(9), Re1–Br1 2.5152(15), C1–O1 1.111(11), C1-Re1-Br1 93.4(3); for **6**: Re1–C11 1.8751(19), Re1–N7 2.1592(14), C11–O11 1.177(2), C11-Re1-N7 94.13(7).

When oxidizing **3** with Fc^+ in acetonitrile (accn), we noticed a color change from blue-green (Fc^+) to orange (Fc) and back to blue-green (Fc^+). This observation indicated a redox-switch like behavior of **3** since Fc^+ reacted first as an oxidant and then, in its reduced form Fc, as a reductant. An-

[a] Dr. F. Zobi, Prof. Dr. R. Alberto
Institute of Inorganic Chemistry, University of Zürich
Winterthurerstrasse 190, 8057 Zürich (Switzerland)
Fax: (+41) 44-635-46-68-03
E-mail: fzobi@aci.uzh.ch

[**] TACN = 1,4,7-triazacyclononane.

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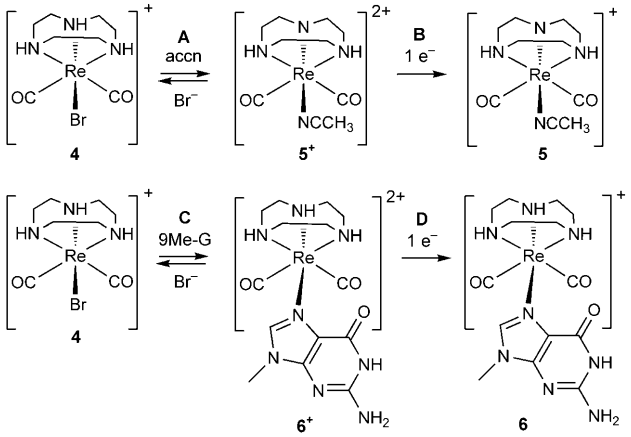
alyzing the solution allowed us to isolate $[Re^{I}(tacn)(accn)(CO)_2]PF_6$ (**5**$[PF_6]$) in 60% yield (see Supporting Information). Obviously, in a first step, Fc^+ oxidized **3** (Re^{I}) → **4** (Re^{II}). Then, the Br^- in **4** was substituted by acetonitrile yielding $[Re^{II}(tacn)(accn)(CO)_2]^{2+}$ (**5**$^+$). This substitution turned the redox switch "on". After substitution of Br^-, the Re^{II} complex **5**$^+$ is a strong oxidant and converted Fc back to Fc^+ whilst being reduced to $[Re^{I}(tacn)(accn)(CO)_2]^+$ (**5**). The irreversible one-e$^-$ wave at $E_{1/2}=+0.78$ V (vs. Ag/AgCl, Supporting Information) rationalized the oxidation of Fc → Fc^+ by **5**$^+$ (Scheme 1).



Scheme 1. Redox switch activity of **4**: Coordination of a substrate (accn in **A** and 9Me-G in **C**) turns the switch "on" since the redox potentials of the resulting complexes **5**$^+$ and **6**$^+$ are strongly increased. Subsequent 1 e$^-$ reduction (**B** and **D**; respectively) results in the highly robust Re^{I} complexes **5** and **6**.

In the net reaction, Br^- in **3** has simply been replaced by accn to give **5**. However, since dissolution of **3** in accn did not lead to even traces of **5**, the conversion described before was not a simple $Br^- \rightarrow NCCH_3$ substitution reaction.

The induction of a strong oxidation potential upon coordination to a target and subsequent reduction to a robust [Re^{I}–substrate] complexes implied a "pro-drug-like" activity for **4**. In contrast to other redox-active complexes, we emphasize that the Re^{II} complex is only reduced after target binding and not before.

Since DNA is a target for inorganic drugs, we probed the "binding–reduction" concept with 9-methylguanine (9Me-G), guanine monophosphate (GMP) and a small oligonucleotide sequence d(5′-CpGpG-3′). When **4** was dissolved in water or MeOH and in the presence of 9Me-G or GMP, the color changed from orange to light yellow. Crystals of $[Re^{I}(tacn)(9Me\text{-}G)(CO)_2]PF_6$ (**6**$[PF_6]$) appeared and an X-ray structure analysis confirmed its authenticity (Figure 1, Supporting Information). The redox potential Re^{I}/Re^{II} of **6**/**6**$^+$ showed one irreversible wave at $E_{1/2}=+0.81$ V. The CVs of **3**/**4** and **6**/**6**$^+$ are compared in Figure 2. The only difference in **4** and **6**$^+$ is one ligand, bromide (**4**) instead of guanine (**6**$^+$). The 600 mV difference between the two complexes caused by the substitution demonstrates the redox switch

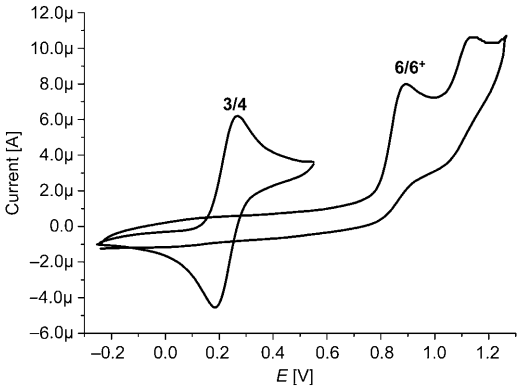
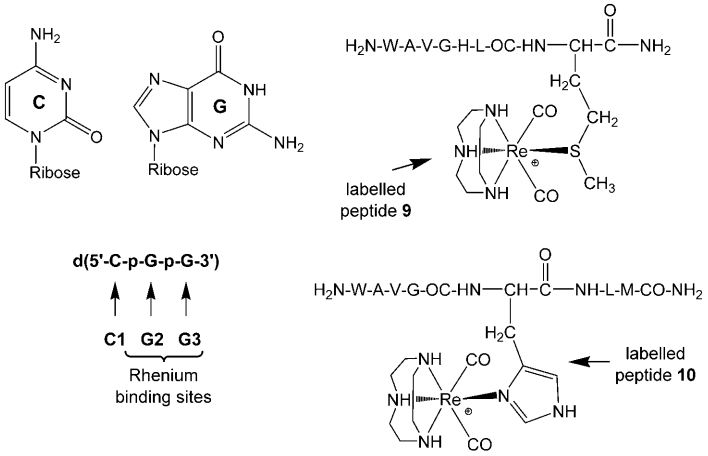


Figure 2. Cyclovoltamograms of the **3**/**4** and the **6**/**6**$^+$ couple. Note the large difference when substituting Br^- for 9Me-G.

concept. Complex **6** and its GMP analogue are very robust in aqueous solution. No decomposition or ligand substitution was observed over days, hence, metalation of guanine was essentially irreversible. The reaction in water with d(5′-CpGpG-3′) yielded two products distinguishable by HPLC-MS in about 30% yield. One corresponded to the coordination of rhenium to G2 and one to G3 (Scheme 2 and Figure 3).



Scheme 2. Reaction products of d(5′-CpGpG-3′) and H_2N-W-A-V-G-H-L-M-$CONH_2$ respectively with $[Re^{II}Br(tacn)(CO)_2]^+$ (**4**) in water.

The induction of reduction by coordination was also found for other potentially coordinating groups in biomolecules. The reaction with histidine (his) or methionine (met) gave $[Re(tacn)(his)(CO)_2]^+$ (**7**) and $[Re(tacn)(met)(CO)_2]^+$ (**8**). For **7** ^{1}H NMR spectra implied coordination to the imidazole nitrogen. Under our conditions, the rate was relatively fast and we found $t_{1/2} \approx 30$ min for all substrates. The reaction of **4** with the peptide H_2N-W-A-V-G-H-L-M-$CONH_2$ in water at RT and under slightly acidic conditions gave the labeled peptide **9** (Scheme 2). Sequential MS^n evidenced that **4** was bound to the methionine side chain. Figure 4 shows the MS spectrum of the parent and the peptide fragments

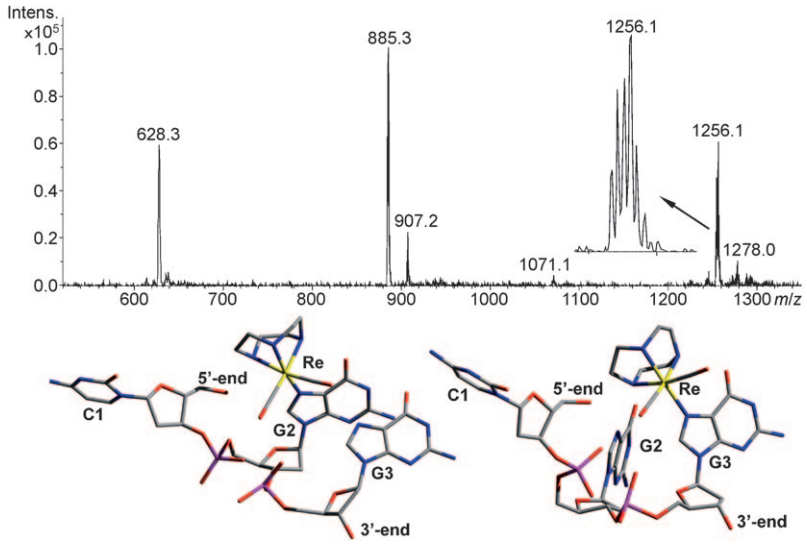


Figure 3. MS spectrum of the labelled oligonucleotide d(5′-CpGpG-3′) and DFT optimized structures of the two adducts formed.

with $[Re(tacn)(met)(CO)_2]^+$ being the last detectable fragment still containing the rhenium isotope pattern. At neutral pH, coordination to histidine was also detected but methionine remained the preferred binding site.

In oligonucleotide sequences or peptides as shown above, the $Re^{II} \rightarrow Re^{I}$ reduction occurred after coordination of **4** since the Re^{I} complex **3** did not at all undergo Br^- substitution by histidine, methionine or guanine. The redox reaction leading to irreversible labelling provoked the question about the reducing agent. For the reaction with 9Me-G, histidine or the peptide, the only reducing agent is Br^- itself, being oxidized to hypobromite $[BrO]^-$. The high $E_{1/2}$ of $\mathbf{6}^+$, the low pH after the reaction as well as an IR band at 620 cm^{-1} were all in agreement with this assumption.[17,18]

When **4** was dissolved in water, the Br^- partly substituted by H_2O under formation of the mono-aqua complex $[Re^{II}(tacn)(OH_2)(CO)_2]^{2+}$ ($E_{1/2} = +0.40$ V, Supporting Information). The substitution of H_2O by, for example, 9Me-G, his or met shifts this potential another 0.4 V more oxidizing. This now turns the switch to "on" and the $\{Re^{II}\}$–L complex is reduced to $\{Re^{I}\}$–L. Since $\{Re^{I}\}$–L is very robust, metalation of L is essentially irreversible. The detailed mechanism is currently underway.

In conclusion, we presented a novel, biologically useful redox-switch based on a water soluble Re^{II} complex. Upon binding to biological sites such as the side chains in peptides or proteins, Re^{II} in, for example, $[Re^{II}(tacn)(his)(CO)_2]^{2+}$ becomes strongly oxidizing and is reduced to the robust Re^{I} complex $[Re^{I}(tacn)(his)(CO)_2]^+$. This reduction leads to essentially irreversible metalation of these biomolecules. Metalation of the peptide H_2N-W-A-V-G-H-L-M-$CONH_2$ exemplified this reactivity. Thus, $[Re^{II}Br(tacn)(CO)_2]^+$ represents an unprecedented example for a biologically relevant redox-switch which is activated after target binding and not before. We are currently studying the reactivity of **4** for site specific introduction of the $[Re^{I}(tacn)(CO)_2]^+$ moiety into proteins.

Experimental Section

Complex $[Re^{II}Br(tacn)(CO)_2]PF_6$ (**4**$[PF_6]$) was synthesized according to the literature.[16] Crystals suitable for X-ray diffraction were grown from an acetonitrile/Et_2O mixture in a glove box. Crystal data for structure **4**$[PF_6]\cdot CH_3CN$: $C_{10}H_{18}Br_1F_6N_4O_2P_1Re_1$, $M = 637.36$, $Z = 4$, orthorhombic, space group *Pnma*, $a = 12.705(4)$, $b = 8.5599(12)$, $c = 16.766(3)$ Å, $V = 1823.4(6)$ Å^3, $T = 183(2)$ K, 20113 reflections measured, $R_1 = 0.0507$ (1450 reflections with $I > 2\sigma(I)$).

Complex $[Re^{I}(tacn)(9Me\text{-}G)(CO)_2]PF_6$ (**6**$[PF_6]$) was synthesized by reacting **4**$[PF_6]$ in water with 2.5 equivalents of 9Me-G. Crystals suitable for X-ray diffraction were collected after two days in 30% yield. Crystal data for structure **6**$[PF_6]$: $C_{14}H_{22}F_6N_8O_3P_1Re_1$, $M = 681.57$, $Z = 2$, triclinic, space group $P\bar{1}$, $a = 7.2764(2)$, $b = 11.4192(2)$, $c = 13.4648(3)$ Å, $\alpha = 76.0070(15)$, $\beta = 88.5846(16)$, $\gamma = 73.5124(15)°$, $V = 1039.80(4)$ Å^3, $T = 183(2)$ K, 36251 reflections measured, $R_1 = 0.0152$ (5908 reflections with $I > 2\sigma(I)$).

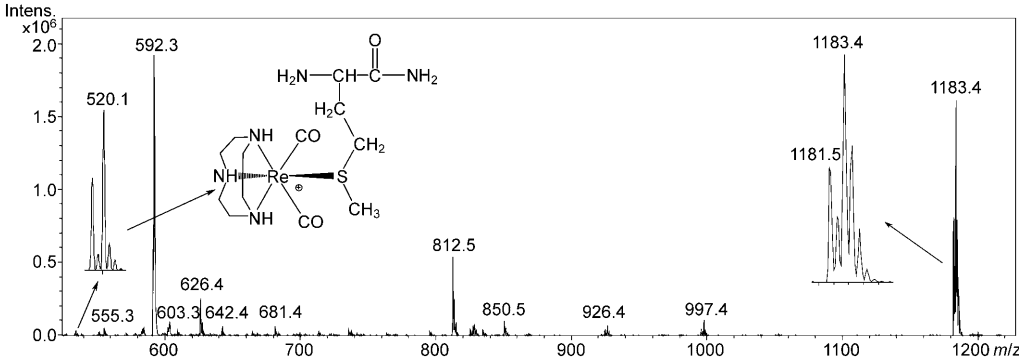


Figure 4. MS spectrum of the labelled peptide **9** and the last fragment $[Re(tacn)(met)(CO)_2]^+$.

CCDC 722654 (**4**[PF_6]) and 722655 (**6**[PF_6]) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

The interaction of **4** with all biological relevant substrates presented in this study, was studied at RT in a 1 M NaBr water solution under a N_2 atmosphere in a wet box. Samples were checked after 1, 4 and 12 h of incubation by HPLC-MS. Yields were determined from the areas of the corresponding peaks. Geometry optimisations for the $[Re^{II}(tacn)(CO)_2]^+$-oligonucleotide adducts shown in Figure 3, were performed at the Density Functional level of theory with the Gaussian03 program package[19] using the hybrid B3LYP functional[20] in conjunction with the LanL2DZ basis set.[21–23] Pure basis functions (5d, 7f) were used in the calculations. Geometries were fully optimised without symmetry restrictions. The G2 and G3 adducts were found to be isoenergetic ($\Delta E = 2.8$ kcal mol^{-1}).

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Keywords: biomolecule · labelling · oligonucleotide · peptide · rhenium

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