The Sal-XH Motif for Metal-Mediated Oxidative DNA−Peptide Cross-Linking

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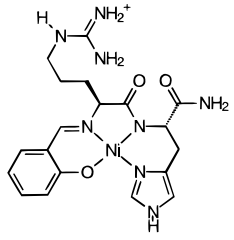
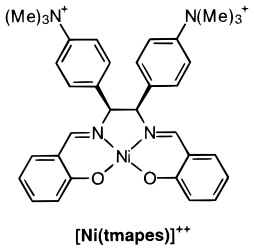
Metallopeptide motifs of the N-terminal sequence XXH, wherein histidine resides in the third position of the peptide, were first described for albumins[1] and later utilized as redox-active bioconjugates for DNA cleavage[2−4] and protein−protein cross-linking.[5] Metallosalens are another class of tetradentate ligands that have seen applications to DNA chemistry.[6−10] The juxtaposition of these two motifs leads to a Schiff-base metallopeptide hybrid that might combine the molecular recognition features of a peptide with the chemical reactivity of salicylaldimine complexes. Toward this end, we describe the synthesis and characterization of a prototypical member of this new "sal-XH" ligand class and an example of DNA−peptide cross-linking.

Bidentate and tridentate Schiff-base adducts to peptides have been previously described, including complexes of a broad range of transition metals.[11,12] These chelates have shown applications as diverse as asymmetric addition of CN^- to aldehydes,[13] epoxides,[14] and imines.[15] The majority of these complexes involve tridentate chelation via the phenolate oxygen, the imine nitrogen, and the first amide bond of the peptide. By including a histidine residue in position 2 of the peptide, we now extend the coordination environment to a square-planar, *tetra*dentate mode while still allowing for C-terminal elaboration of the peptide.



[Ni(sal-RH)]$^+$

1



[Ni(tmapes)]$^{++}$

2

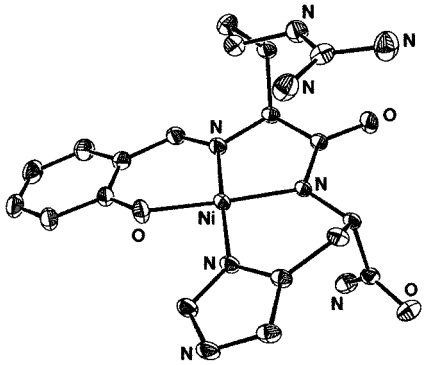


Figure 1. ORTEP plot of [Ni(sal-RH)]$CH_3CO_2 \cdot 3H_2O$, **1**; the hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (deg): Ni−O(1), 1.8555(14); Ni−N(1), 1.8576(14); Ni−N(2), 1.8721(15); Ni−N(3), 1.8931(14); N(1)−C(7), 1.285(3); N(1)−C(8), 1.484(2); N(2)−C(9), 1.337(2); N(2)−C(10), 1.459(2); N(5)−C(15), 1.327(3); O(1)−Ni−N(1), 94.05(7); O(1)−Ni−N(2), 177.08(8); N(1)−Ni−N(2), 85.14(7); O(1)−Ni−N(3), 87.91(7); N(1)−Ni−N(3), 177.54(7); N(2)−Ni−N(3), 92.98(7).

Arginine (R) was chosen for position "X" of the sal-XH motif in order to impart water solubility and some degree of DNA affinity to the complex. [Ni(sal-RH)]OAc, **1**, was synthesized by addition of bis-(salicylaldehyde)Ni to a pH 8, 1:2 ethanol/water solution of the dipeptide, Arg-His bearing a C-terminal carboxamide. Orange microcrystals were obtained from this solution after 4 h at room temperature; crystals suitable for X-ray crystallographic analysis were obtained by recrystallization from 2:1 methanol/water. The crystal structure of **1**, shown in Figure 1, depicts the tetradentate nature of the sal-RH ligand as predicted.[16] The coordination geometry about the nickel ion is square-planar as evidenced by the very small deviation (0.001 Å) from the plane defined by the four ligand donor atoms. The overall charge on the molecule is positive due to the guanidino moiety of arginine; an acetate counterion is present in a hydrogen-bonded network of three water molecules associated with the periphery of the ligand.

Consistent with the crystal structure, the solution structure of [Ni(sal-RH)]$^+$ appears to be that of a square-planar, diamagnetic species based on the NMR and UV−visible absorption spectra (d−d transition at 398 nm, $\epsilon = 1930$ cm^{-1} M^{-1}). Cyclic voltammetric studies of **1** as a 1 mM aqueous solution (pH 7, 10 mM NaP_i, 100 mM NaCl) showed a largely irreversible oxidation at $E_p \approx +0.85$ V vs Ag/AgCl (scan rate = 100 mV s^{-1}), although some evidence for a small reduction at 0.80 V could be detected ($i_{pc}/i_{pa} \approx 0.4$). This behavior is consistent with ligand-centered oxidation due to the facile formation of a phenolate radical, as is typically observed in Ni(salen) complexes.[11,17]

The ligand-centered redox chemistry of the new Ni(sal-XH) complex immediately suggested that it might participate in an oxidative cross-link with DNA. We previously reported that the water soluble salen complex, Ni(tmapes), **2**, formed an adduct to guanine residues under oxidative conditions.[6,18,19] In those studies, treatment of DNA or RNA with **2** in the presence of $KHSO_5$ led to the formation of a guanine adduct that could be characterized by electrospray ionization MS (ESI-MS),[20] primer extension

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(16) Crystal data (293 K) for [Ni(sal-RH)]OAc·$3H_2O$, **1**: $NiC_{21}H_{34}N_8O_8$, $M_r = 585.27$, monoclinic, space group *C*2, $a = 17.0767(3)$ Å, $b = 9.1342(3)$ Å, $c = 16.3666(5)$ Å, $\alpha = 90°$, $\beta = 101.4810(16)°$, $\gamma = 90°$, $V = 2501.81(12)$ Å^3, $Z = 4$, GOF = 1.089. Final *R* values ($I > 2\sigma I$): $R1 = 0.0334$, $wR2 = 0.0969$.
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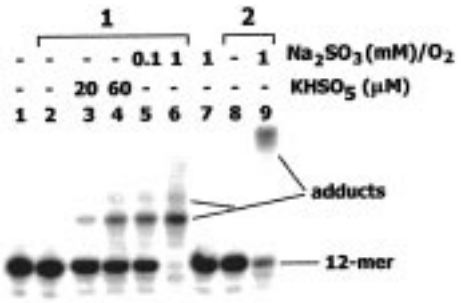

Figure 2. Phosphorimage of 20% polyacrylamide denaturing gel (7 M urea) showing high-molecular weight adducts to DNA formed with nickel complexes. The 12-mer oligodeoxynucleotide, 5′-d(ATATCAGATCTA)-3′, was labeled at the 5′ terminus with ^{32}P and incubated with reactants under the standard conditions described in Supporting Information. Lane 1: DNA alone. Lane 2: control with 100 μM Ni(sal-RH) only. Lanes 3−6: 100 μM **1** plus the indicated concentrations of oxidant. Lane 7: control showing the effect of Na_2SO_3 without metal complex. Lane 8: control with 2 μM **2**. Lane 9: 2 μM **2** plus Na_2SO_3. All experiments were carried out in the presence of air and included EDTA in the workup. Cross-linking yields in lanes 3, 4, 5, and 6 were 16, 36, 39, and 80%, respectively.

assays,[21] and PAGE analysis of short oligonucleotides.[18] The participation of the phenolate moiety in **2** as part of the cross-link was confirmed through substituent effect studies,[20] and the modified guanine was found to be a piperidine-labile DNA cleavage site.[18,20]

Studies reported herein show that the cross-linking behavior of a phenolate moiety can now be incorporated into the N-terminal motif of a peptide. As shown in Figure 2, treatment of a 5′-[^{32}P]-end-labeled 12-mer oligodeoxynucleotide with **1** (100 μM) and $KHSO_5$ (20 or 60 μM, lane 3 or 4), followed by quenching with HEPES + EDTA, led to a higher molecular weight band observable by PAGE. Interestingly, **1** appears to share with nickel peptides[22] and nickel salens the ability to catalyze HSO_5^- formation in situ via autoxidation of sulfite. Thus, cross-linking of **1** to DNA was even more effective when $KHSO_5$ was replaced with Na_2SO_3 (0.1 or 1 mM, lane 5 or 6). Sulfite can be used in greater concentration than HSO_5^-, an oxidant that would lead to indiscriminant background oxidation of DNA if used in concentrations >100 μM. Control studies confirmed that no high-molecular weight band was observed if Ni^{2+} + RH (lacking salicylaldehyde) was used in place of **1**. The positions on the gel of the high-molecular weight bands from **1** are significantly lower than that from **2** (whose DNA cross-link is shown for comparison in Figure 2, lane 9),[23] suggesting that the charge and molecular weight of this adduct are different.

Treatment of the [Ni(sal-RH)]$^+$-modified DNA with 0.2 M piperidine at 90 °C for 30 min led to substantial cleavage, but only at the single G residue of the oligomer (see Supporting Information).[24] Control studies demonstrated that no other base modifications, such as imidazolone formation,[25] were produced by these reaction conditions. The piperidine sensitivity of the DNA adduct suggests that the cross-link has likely formed to either N7 or C8 of G, since both of these sites of modification typically lead to piperidine-induced strand scission.[19] Indeed, arylation of guanine at both of these positions has been observed for the phenolic A-ring of estrogen.[26] Mass spectral analysis provided further information on the site and identity of adduct formation; isolation of the major **1**•DNA adduct from the gel and precipitation with NH_4OAc led to a sample suitable for ESI-MS. The final spectrum showed a single product of mass 3739 consistent with DNA + salicylaldehyde − 10 Da (see Supporting Information). The overall loss of 10 mass units corresponds to the now generally observed phenomenon of further oxidation of any C8-modified guanine adduct leading to a guanidinohydantoin product after hydration and decarboxylation of C6 of the purine.[27−29] Thus, the DNA partner in cross-link formation appears to be C8 of G, and the imine bond has hydrolyzed due to the presence of EDTA in the workup. A higher, faint band visible in lane 6 of Figure 2 was also analyzed by ESI-MS and found to correspond to the intact nickel complex **1** adducted to the guanidinohydantoin moiety of the DNA strand (i.e., DNA + **1** − 10 Da = 4089 Da). This band is more pronounced if EDTA is omitted from the quenching step. On the sal-RH ligand, the positions ortho and para to the phenol oxygen are suspected sites of reactivity based on inhibition of cross-linking when these sites are substituted in **2**.[20] Final confirmation of the structure of the adduct awaits large-scale preparation and X-ray or NMR characterization.

In summary, the new hybrid ligand sal-XH, as demonstrated in the prototype complex **1**, combines the chemical reactivity of a salen complex with the potential molecular recognition properties of a peptide. In principle, any protein or peptide containing histidine as the second residue (and not containing proline as the N-terminal residue) may now be converted to a DNA or RNA cross-linking agent. After hydrolysis of the imine linkage, one obtains a nucleic acid specifically derivatized with a salicylaldehyde moiety at the cross-link site. This unique aldehyde might be further conjugated to fluorescent or biotinylated probes for analysis of peptide/protein binding sites in DNA or RNA. Such applications, as well as extensions to other transition metals are currently underway.

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Supporting Information Available: Experimental procedure for preparation and characterization of **1**, ESI-MS data, PAGE analysis showing control studies and piperidine treatment (PDF) and X-ray crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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