

Formation of DNA adducts using nickel(II) complexes of redox-active ligands: a comparison of salen and peptide complexes

James G. Muller ^a, Lou Anne Kayser ^a, Sari J. Paikoff ^b,
Victor Duarte ^a, Ning Tang ^{b,c}, Ronelito J. Perez ^a,
Steven E. Rokita ^{b,c}, Cynthia J. Burrows ^{a,b,*}

^a Department of Chemistry, University of Utah, Salt Lake City, UT 84112, USA

^b Department of Chemistry, State University of New York, Stony Brook, NY 11794, USA

^c Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20792, USA

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* Corresponding author. Tel.: +1-801-585-7290; fax: +1-801-585-7868.

E-mail address: burrows@chemistry.utah.edu (C.J. Burrows)

Abstract

A series of water-soluble Ni(salen) complexes have been synthesized and studied under oxidative conditions with DNA. Experiments involving gel electrophoretic analysis, substituent effect studies, and mass spectral characterization point to the formation of covalent adducts of the salen ligand with guanine nucleobases. The mechanism of this novel reactivity of transition metal complexes with nucleic acids is discussed in comparison with nickel(II) complexes of peptides and other ligands. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Nickel; Salen; Peptides; DNA; Oxidation

1. Introduction

The selective modification of DNA and RNA by transition metal complexes has blossomed into a rich field of research [1,2]. A number of different approaches may now be taken toward oxidation or hydrolysis of the sugar-phosphate backbone of nucleic acids using transition metal and lanthanide species [3,4]. Similarly, nucleobase-targeted oxidation using redox-active metals has enjoyed a great deal of attention recently [5]. Binding of metal complexes to nucleic acids typically takes advantage of either direct metal–base bonds (such as Pt–G–N7) or non-covalent interactions such as π stacking and hydrogen bonding of the organic ligands of a metal complex to DNA's grooves or base pairs [1]. In contrast, little attention has been focused on covalent modification of nucleobases with metal complexes. In this scheme, the coordinated metal ion can serve to trigger redox chemistry that is *ligand-based*, generating a reactive intermediate that couples with nucleobases to form a new covalent bond (Fig. 1). Such covalent modification of DNA (or RNA) can be directed toward a variety of chemical and biological goals: probing nucleic acid structure, understanding DNA reactivity, regulating gene expression, or triggering apoptosis via chromosome modification.

Nickel(II) complexes based on the salen ligand (where salen = ethylenediamine-*N,N'*-bis(salicylalimine)) are well suited for covalent modification of DNA since the $\text{Ni}^{\text{III/II}}$ couple often lies near the redox potential of the ligand, at ca. 1 V versus SCE. In addition, the irreversibility of the cyclic voltammograms of most nickel(II)-salen-type complexes suggests that the one-electron oxidation is ligand-based and

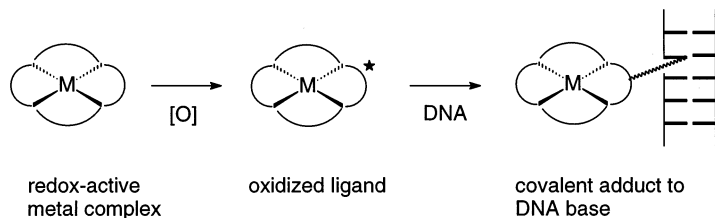


Fig. 1. General scheme for oxidative alkylation of nucleobases by redox-active metal complexes.

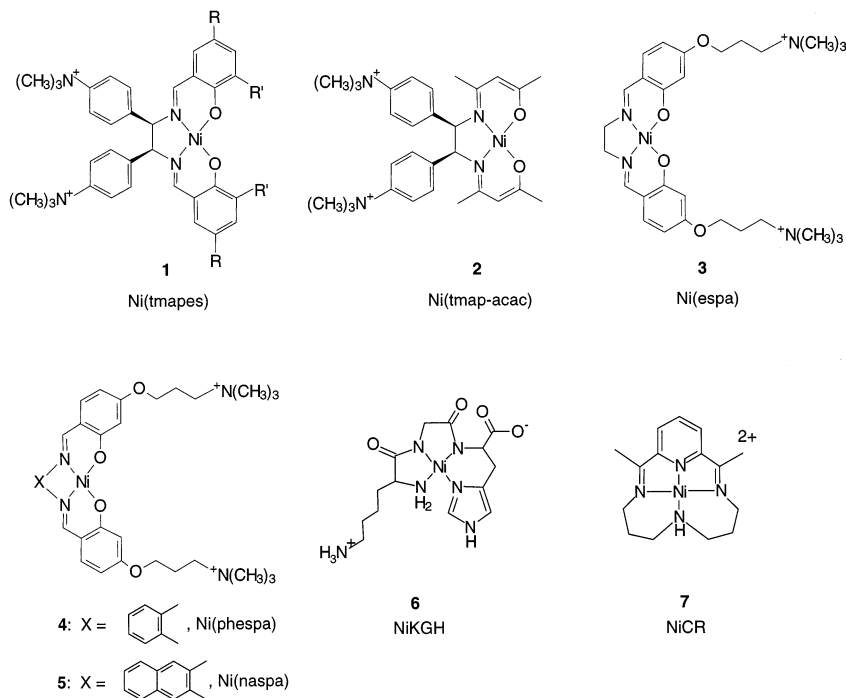


Fig. 2. Structures of nickel(II) complexes reviewed in this paper. (R, R' = H, Me, Cl).

leads to phenolic radicals, capable of polymerization via C–C bond formation between the ortho and *para* position of the phenol moiety [6,7]. One can then imagine that ligand radical intermediates, when bound to DNA, might be intercepted by reactive groups on nucleobases leading to covalent modification. This review describes our recent efforts toward this goal and compares the results of DNA modification using salen-type complexes (Fig. 2, complexes 1–5) with other nickel complexes studied in our laboratories (Fig. 2, complexes 6 and 7).

2. Synthesis of water-soluble salen complexes

Our previous use of nickel-salen complexes as catalysts for olefin epoxidation was not directly applicable to DNA modification for reasons of water insolubility [8,9]. Thus, we sought water-soluble analogs of salen that would generate square-planar nickel(II) complexes bearing an overall positive charge for affinity towards nucleic acids. Two different approaches were taken; one involved placing quaternary ammonium groups on the ethylenediamine unit (1), and the second utilized quaternary ammonium-functionalized salicylaldehydes (3–5). Alternatively, two other cationic salen ligands have been reported in the literature recently. One is based on a pendant alkylammonium group on the ethylene diamine moiety and

thus carries an overall charge of +1 [10], and the other places ammonium substituents in the *para* position of the salicylaldehyde groups [11], which we would predict (*vide infra*) would inhibit DNA adduct formation.

2.1. Tmapes (**1**) and its derivatives

The preparation of *meso*-1,2-bis(4-trimethylammoniumphenyl)ethylenediamine-*N,N'*-bis(salicylalimine), or 'tmapes' was based on a synthesis of the parent ligand, dmapes, containing dimethylaminophenyl substituents on the ethylenediamine group reported by Vögtle and Goldschmitt [12]. Details of the synthesis of **1a** ($R, R' = H$) have already been reported by us [13]. In order to probe the mechanism of oxidative coupling to DNA, we also synthesized a series of derivatives: **1b**, $R = CH_3$, $R' = H$; **1c**, $R = H$, $R' = CH_3$; **1d**, $R = R' = CH_3$; **1e**, $R = Cl$, $R' = H$; **1f**, $R = R' = Cl$, using the general scheme outlined in Fig. 3. In this procedure, acid-catalyzed hydrolysis of the Schiff base ligand dmapes followed by neutralization with NaOH permitted isolation of the functionalized ethylene diamine, dmape. Formation of a new salen ligand was accomplished by addition of the desired salicylaldehyde, followed by coordination of nickel(II) using its acetate salt. While in principle it would have been possible to synthesize a substituted dmapes ($R \neq H$) using a procedure analogous to that previously published [12], it was higher yielding to obtain the diamine dmape from hydrolysis of dmapes and then condense with a

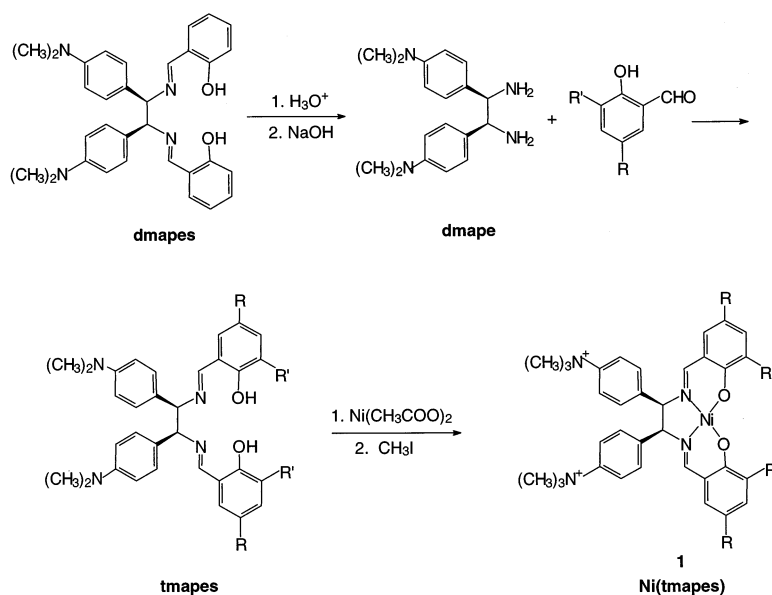


Fig. 3. Synthetic scheme for tmapes complexes.

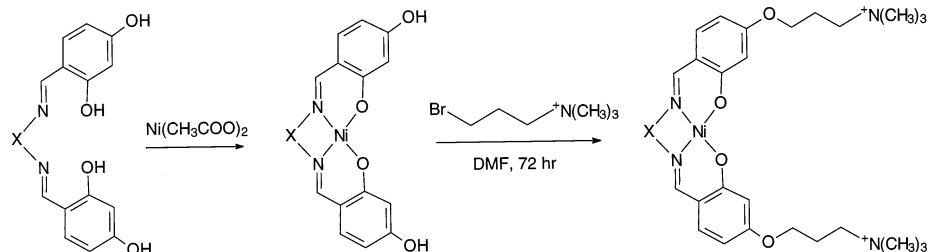


Fig. 4. Synthetic scheme for espa-type complexes.

new, substituted salicylaldehyde. In a final step, the quaternary ammonium groups were prepared by alkylation with CH_3I . All of the complexes of type **1** were square-planar, diamagnetic complexes that gave satisfactory analytical data. For comparison, complex **2** was synthesized using acetylacetonate in place of the salicylaldehyde [13].

2.2. *Espa (3) and its derivatives*

Because the synthesis of the functionalized diamine, dmape, was somewhat cumbersome, we sought an alternative salen-type ligand with positively charged groups in a position that would be synthetically convenient while not hindering DNA adduct formation, which we postulate to involve the *ortho* and *para* positions of the phenolate group. The salen ligand with propylammonium groups on the 4-position of the salicylaldehyde moiety (*espa*) appeared attractive. This same ligand was prepared concurrently by Mandal, et al. [14]; our synthesis is somewhat different as outlined in Fig. 4.

The first Schiff base ligand in this series was prepared using ethylenediamine and 2,4-dihydroxybenzaldehyde by refluxing the two components in ethanol for 30 min. Subsequent treatment of the precipitate with $\text{Ni}(\text{OAc})_2$ in propanol at 100°C led to formation of the complex as an orange solid. Reaction with 3-bromopropyltrimethylammonium bromide and K_2CO_3 in DMF led to bis-O-alkylation of the 4-hydroxy substituents selectively, since the 2-hydroxyls were protected by coordination to nickel(II). This compound was purified by precipitation from an aqueous 1 M solution of NaClO_4 , and then stored as the chloride salt after ion exchange chromatography using Dowex chloride resin. Analogs **4** and **5** were prepared similarly using *o*-phenylenediamine and *o*-naphthalenediamine, respectively, in place of ethylenediamine. All intermediates and final complexes were completely characterized by IR, ^1H -NMR, ^{13}C -NMR, UV-vis spectroscopy and MS [15]. Complexes **3–5** were orange or red-orange with characteristic d–d transitions at about 430 nm as expected for square-planar complexation.

3. DNA modification with nickel(II)-salen complexes

Metallosalen complexes capable of DNA modification include manganese(III) [16,17], cobalt(II) [18–20], nickel(II) [10,13,14,20,21], and copper(II) [22–26] compounds. Overall, it appears that Mn, Co, and Cu complexes in the presence of oxidants ranging from O_2 to peracids lead to direct strand scission of DNA, presumably due to hydrogen atom abstraction from a deoxyribose group. Nickel-salen complexes are unique in their properties, in that they show much higher preference for modification of guanine residues, and strand scission is only observed after piperidine treatment. We believe this is due to the formation of a salen-guanine adduct according to the evidence provided below.

3.1. Reactions with synthetic oligodeoxynucleotides

Initial studies were carried out with a synthetic oligodeoxynucleotide that formed a stable hairpin, namely: 5'-d(AGTCTATGGGTTAGACT)-3', where the underlined residues are present in a single-stranded loop region. Treatment of a 3- μ M buffered solution (10 mM NaP₆, 100 mM NaCl) of DNA with 3 μ M Ni(tmapes) (**1a**) and 10 μ M KHSO₅ led to no observable cleavage bands (reaction time = 30 min.), but instead showed a higher molecular weight species of slower elec-

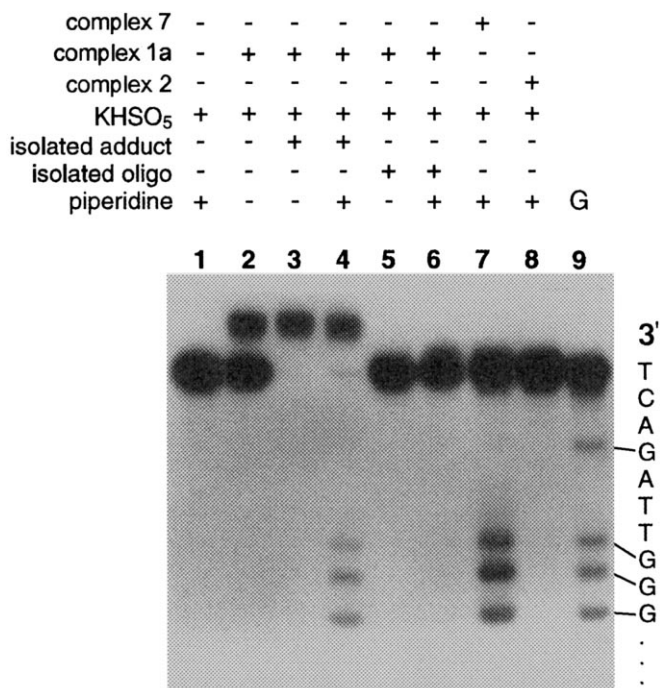


Fig. 5. PAGE analysis of DNA adducts formed by Ni(tmapes), **1a** + KHSO₅. See Ref. [13] for details.

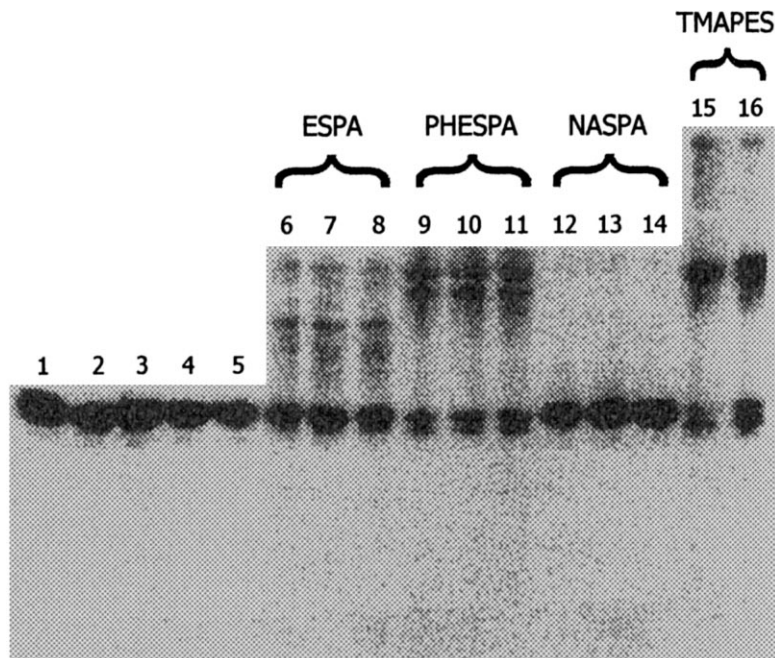


Fig. 6. PAGE analysis of DNA adducts formed by nickel complexes + KHSO_5 . Experimental protocols were similar to those described for Fig. 5 [13]. Lanes 1–5: controls with DNA + 120 μM KHSO_5 only (1), 2 μM **3** (2), 6 μM **4** (3), 60 μM **5** (4), 2 μM **1**, (5). Lanes 6–8: DNA + 2 μM **3** + 30 μM KHSO_5 . Lanes 9–11: DNA + 6 μM **4** + 120 μM KHSO_5 . Lanes 12–14: DNA + 60 μM **5** + 120 μM KHSO_5 . Lanes 15–16: DNA + 2 μM **1** + 30 μM KHSO_5 .

trophoretic mobility (Fig. 5, lanes 2–4) [13]. When the upper band was isolated from the gel (Fig. 5, lane 3) and treated with hot piperidine (0.2 M, 90°C, 30 min.), some of the material led to strand scission at the exposed Gs of the hairpin loop (Fig. 5, lane 4). More extensive treatment with piperidine, up to 2.5 h, showed that about 80% of the high molecular-weight adduct is a piperidine-labile guanine species. Interestingly, no simple guanine oxidation leading to piperidine-induced cleavage was observed with Ni(tmapes) (Fig. 5, lanes 5,6) in contrast to the typical chemistry seen with other nickel(II) complexes such as NiCR. Complex **2**, derived from acetylacetone and lacking a phenolic moiety, showed no adduct formation under similar conditions (Fig. 5, lane 8), and NiCR, **7**, yielded only piperidine-labile guanine oxidation without adduct formation (Fig. 5, lane 7).

Analogous studies were carried out with Ni(espa), Ni(phespa) and Ni(naspa) (**3–5**), and the results are shown in Fig. 6 and compared to Ni(tmapes). Both Ni(espa) (Fig. 6, lanes 6–8) and Ni(phespa) (Fig. 6, lanes 9–11) behave similarly to Ni(tmapes) (Fig. 6, lanes 15–16) in producing high molecular weight bands seen by gel electrophoresis after reaction with a 12-mer oligodeoxynucleotide plus KHSO_5 as oxidant. Curiously, Ni(naspa) was unreactive with DNA in the presence of oxidant (Fig. 6, lanes 12–14). Whether this is due to different redox chemistry

occurring with the naspa ligand or due to a different mode of interaction with DNA, possibly intercalative, is not known.

3.2. Primer extension analysis of large DNA and RNA targets

The studies with short oligodeoxynucleotides described in the previous section point to formation of a higher molecular weight DNA species when nickel(II)-salens are oxidized by monoperoxysulfate. It is important to note that the high molecular-weight band can only be resolved with short strands of DNA; the study of restriction fragments of lengths typically >100 nucleotides would lead to an imperceptible change of a band on a gel. The fact that most of the material is converted to a strand scission site at exposed guanines suggests that most, perhaps all, of the modification sites are at Gs. To confirm this, various primer extension studies were carried out. In this assay, a long fragment of DNA (or RNA) is chemically modified by a nickel complex + KHSO_5 . A short, radiolabeled primer that is complementary to a region immediately 3' to the area of interest on the target is annealed and extended in the presence of a polymerase and dNTPs. When this study was carried out with an RNA target, namely the L-21 Sca Group I intron from *Tetrahymena*, all of the sites of modification were found to be exposed guanine residues [27].

In order to confirm this finding with DNA, primer extension studies were carried out with both single-stranded and double-stranded DNA targets. M13mp18 is a 7.2 kb single-stranded DNA plasmid. After it was allowed to react with 50 or 500 nM Ni(tmapes) (Fig. 7, lanes 3 & 4, respectively) and 50 μM KHSO_5 , a complementary primer was extended and sequenced. The stop points corresponded primarily to G residues, although modification of one T is apparently seen (Fig. 7, lane 4). When double-stranded pBR322 was used as the target of modification, very little reaction was observed confirming the higher reactivity of guanines exposed in single-stranded regions [28].

3.3. Substituent effects on DNA alkylation

In order to gain more insight into the nature of the high molecular-weight adduct formed between DNA (or RNA) and Ni(salen) complexes, a series of substituted tmapes ligands were studied in which electron-donating (CH_3) and electron-withdrawing (Cl) groups were positioned *ortho* and *para* to the phenolate oxygen (**1b–f**). The extent of DNA adduct formation was monitored as the intensity of the high molecular-weight band on an electrophoretic gel compared to the band corresponding to unmodified DNA. These are plotted as a function of substituent in Fig. 8. Methylation of the *ortho* or *para* positions of the phenol individually had a small but noticeable effect. Methylation of both positions, **1d**, diminished the amount of adduct formation by more than half, but did not eliminate it completely, as is seen for the *o,p*-dichlorophenol-containing ligand, **1f**.

These data are consistent with the hypothesis that an adduct is formed between the *ortho* or *para* position of the salen ligand and a site on a guanine nucleobase.

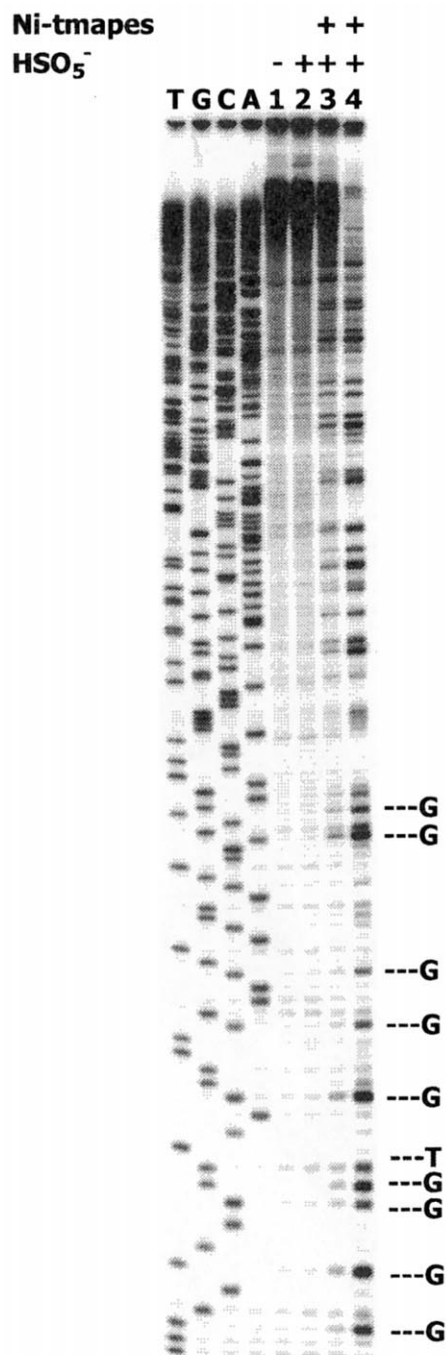


Fig. 7. PAGE analysis of an extended primer after modification of M13mp18 with **1a** and KHSO₅. Sanger sequencing lanes are labeled T, G, C, and A. Lanes 1 and 2 are control experiments that were performed without **1a** in the absence or presence, respectively, of 50 μ M KHSO₅. Lanes 3 and 4 represent experiments with 50 μ M KHSO₅ and 50 or 500 nM, respectively, of **1a**.

One would expect unpaired electron density to reside at these positions if the phenolate portion of the ligand is responsible for one-electron oxidation of the complex, as is implied by the unreactivity of the acetylacetonate complex **2**. Substitution with chlorine at the *ortho* and *para* positions as in **1e** and **1f** would be expected to diminish and eliminate, respectively, adduct formation, as is observed. The fact that methyl substituents do not completely inhibit adduct formation is consistent with the reported ability of arene radicals bearing methyl substituents to exhibit unpaired electron density on the exocyclic carbon after hydrogen atom abstraction from the methyl group [29]. Thus, complexes **1b–d** have an alternative site for radical formation and covalent bond formation to guanine.

3.4. Characterization of adducts by ESI-MS

In order to confirm that the high molecular-weight band observed on electrophoretic gels is indeed a DNA-salen adduct, the material representing this band from a reaction of a synthetic 12-mer, 5'-d(ATATCAGATCTA)-3', with Ni(tmapes), **1a**, was isolated from the gel, and purified by precipitation from NH₄OAc to remove K⁺ and Na⁺ [30]. The mass spectrum of this material using electrospray ionization methods is shown in Fig. 9. The observed mass for the unreacted oligodeoxynucleotide is 3628 (data not shown) [30]. If adduct formation occurs with subsequent re-aromatization of both the phenolic moiety and the nucleobase, one would expect the adduct to appear at a mass of 4218, consistent with the smaller peak shown in Fig. 9. The major peak at 10 mass units lower than anticipated is difficult to explain, but may be due to loss of a methyl group from

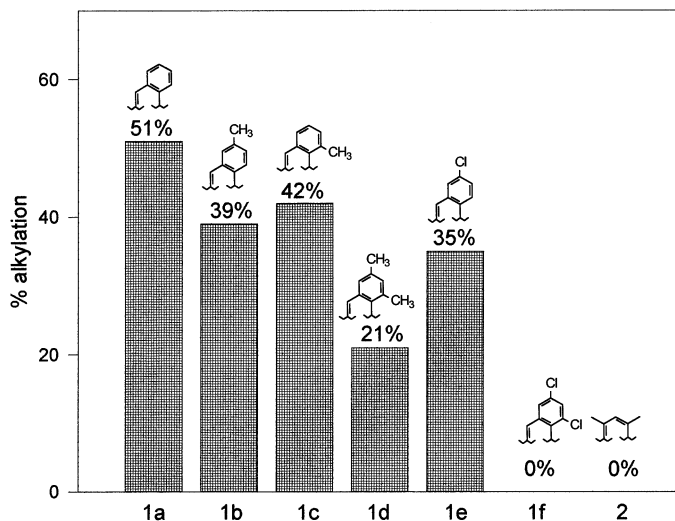


Fig. 8. Extent of DNA-adduct formation with various Ni(tmapes) analogues as measured by the intensity of the high molecular-weight band observed by gel electrophoresis using a synthetic oligomer.

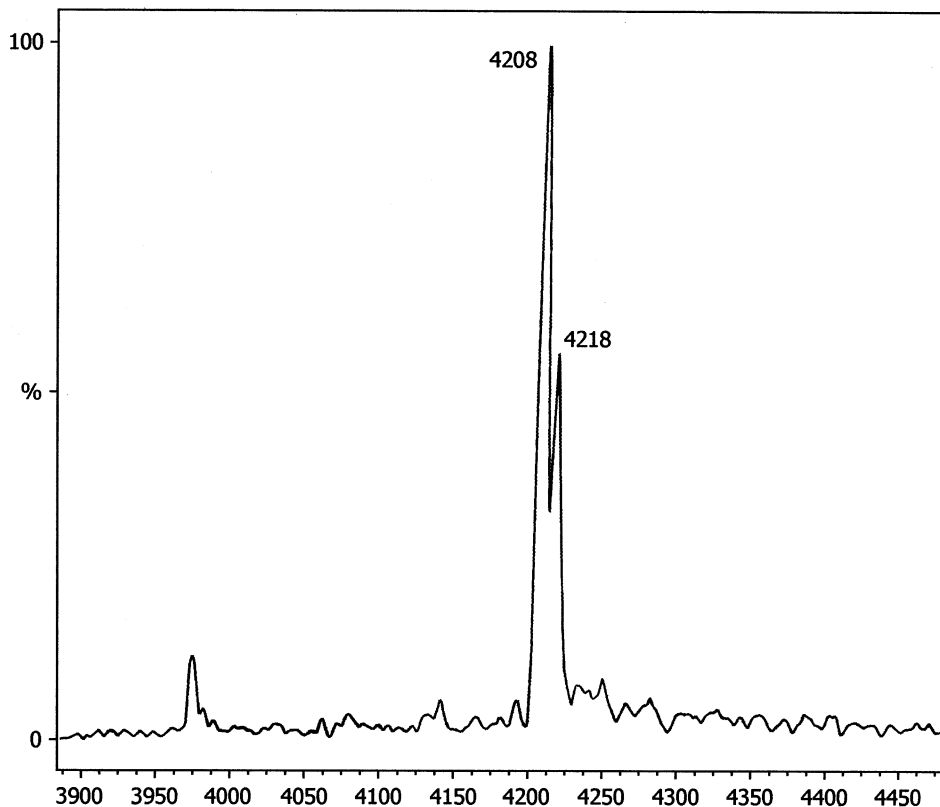


Fig. 9. ESI-MS spectrum of high molecular-weight DNA adduct isolated from the reaction of a synthetic 12-mer (see text), complex **1a**, and KHSO_5 .

a quaternary ammonium site. High molecular-weight materials obtained from reactions with $\text{Ni}(\text{espa})$ and $\text{Ni}(\text{phespa})$ gave similar results.

4. Comparison with metal peptide chemistry

A second example of covalent adduct formation between a nickel complex and DNA under oxidative conditions also comes from our laboratory. The N-terminal, tripeptide motif, XXH , is known to be a good binding site for nickel(II) and copper(II) in proteins and peptides [31]. Furthermore, the aerobic oxidation of these complexes bearing a free carboxylate C-terminus has been described [32–34]. We found that DNA was able to intercept a reactive intermediate formed during nickel peptide autoxidation to form a covalent adduct using complex **6** [30,35].

Superficially, these two systems— $\text{Ni}(\text{salen})$ and $\text{Ni}(\text{peptide})$ adduct formation with DNA—appear to be related. Both require oxidation of the ligand, and both lead to covalent adduct formation with the nucleobase. However, the adducts have

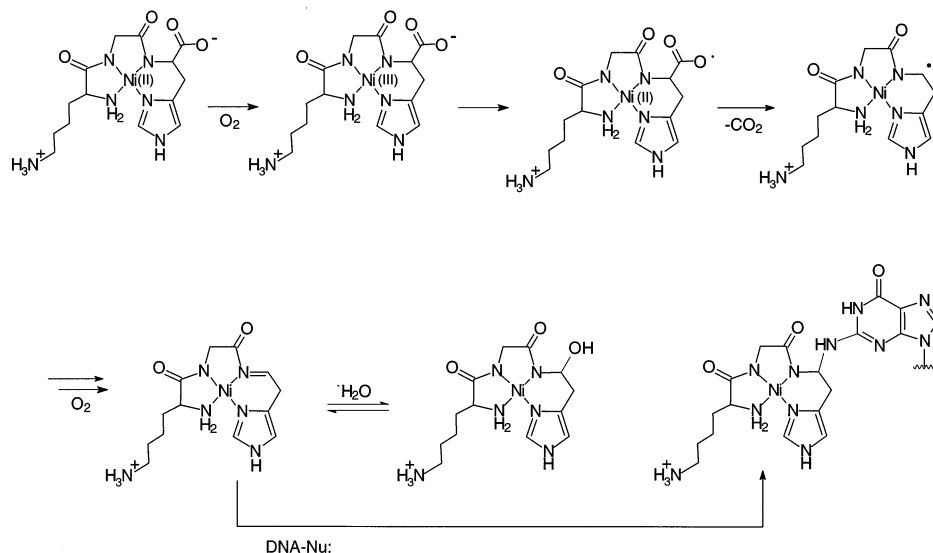


Fig. 10. Proposed mechanism of nucleobase modification with redox-active nickel peptides (6).

quite different properties, and we propose different mechanisms for the two reactions. For example, the Ni(peptide) adduct is not piperidine labile while the Ni(salen) adducts are. Ni(salen) adducts occur almost exclusively at guanine residues, while Ni(peptide) adducts show a preference for G, but are less base-specific. The difference most likely rests with the relatively high stability of phenolic radicals compared to peptide radical intermediates. A mechanism for Ni(peptide) alkylation of DNA is shown in Fig. 10 [5]. Although an intermediate carbon radical is likely formed during oxidative decarboxylation, it should be rapidly further oxidized to an imine. The resulting N-acylimine should be a good trap for nucleophiles present on all of the DNA bases. Such π -type electrophiles often react with the exocyclic amino group of guanine; alkylation of this site does not lead to a piperidine-labile lesion.

The behavior of Ni(salen)-type complexes is best explained as the addition of a phenol radical to the guanine heterocycle (see Fig. 11). Oxidation of Ni(tmapes) is expected to be principally based on the phenolate group leading to unpaired electron density on the *ortho* and *para* carbons (with respect to the phenol oxygen). Radical addition to C8 of guanine is very well precededented; examples include $HO^\bullet$ [36], $H_3C^\bullet$

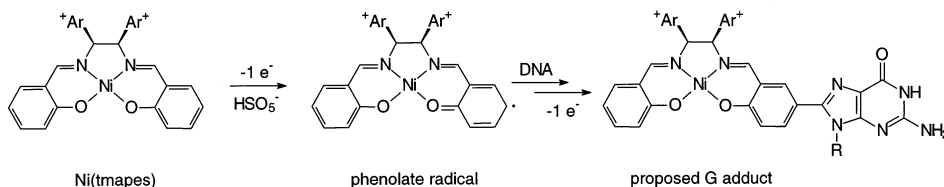


Fig. 11. Proposed mechanism of guanine modification with Ni(salen)s.

[37], benzylic radicals [29], and estrogen radicals [38,39], the latter being a phenol radical. Alkylation (or arylation) of guanine at C8 is a piperidine-labile site [5]. We therefore propose that the major adduct formed between Ni(salen)s and guanine involves covalent bond formation between the *ortho* or *para* carbons (or exocyclic methylene for **1b–d**) and C8 of guanine. Other alkylation sites are possible, especially on guanine, including N7 and N², the latter perhaps accounting for non-piperidine-labile adduct formation.

5. Conclusions

Studies of new, water-soluble Ni(salen)-type complexes with DNA under oxidative conditions have been carried out using gel electrophoretic experiments, examination of substituent effects, and mass spectral characterization of adducts. The results point to an efficient coupling process in which the redox activity of the nickel complex is ligand-centered, leading to a phenol radical capable of covalent modification of guanine nucleobases, particularly in single-stranded DNA and RNA. Although studies of water-soluble Ni(salen)s in other laboratories have emphasized the piperidine-induced strand scission [10] or polymerase ‘stops’ [14] mediated by these complexes, we feel that the actual mechanism involving covalent adduct formation is the novel aspect of this interesting system. This new mechanism now opens up interesting avenues for the design of nucleic acid-targeted metal complexes with specifically tailored properties.

Acknowledgements

Support of this work by grants from the National Institutes of Health (GM-49860 to CJB and GM-47531 to SER) is gratefully acknowledged. We also thank R.P. Hickerson for technical assistance.

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