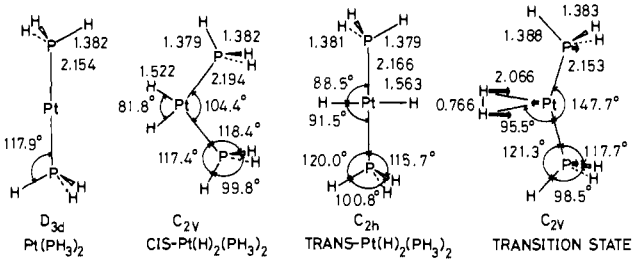


**Figure 1.** Fully optimized geometries (in Å and deg) of $Pt(PH_3)_2$, *cis*-$Pt(H)_2(PH_3)_2$, *trans*-$Pt(H)_2(PH_3)_2$, and the transition state. Arrows in the transition state show the reaction coordinate vector.

Table I. Calculated Energy Profile Relative to $Pt(PH_3)_2 + H_2$ (in kcal/mol)

| method | transition state | *cis*-$Pt(H)_2(PH_3)_2$ | *trans*-$Pt(H)_2(PH_3)_2$ |
|---|---|---|---|
| RHF | +5.2 | −36.9 | −38.0 |
| CI | +8.7 | −27.0 | −25.1 |
| CI + QC[a] | +7.1 | −27.0 | −24.2 |
| CI + QC + ZPC[a] | +8.2 | −21.7 | −20.5 |

[a] QC, correction for unlinked quadruple excitations (Davidson, E. R.; Silver, E. W. *Chem. Phys. Lett.* 1977, *52*, 403). ZPC, correction for zero-point energy.

transition state where the HH bond is stretched only 4%.

Calculations were performed for a singlet, the ground state according to experimental evidences.[1-3] We optimized all the degrees of freedom by using the energy gradient[5] at the restricted Hartree–Fock (RHF) level under the relativistic effective core potential approximation.[6] A smaller basis set (valence double except for the $PH_3$ part)[7] was used for calculating structures and normal modes and a larger set (all valence double)[7] for energies. To obtain better energetics, configuration interaction (CI) calculations were carried out at RHF optimized geometries with the larger basis set, including all the single and double excitations relative to the RHF configuration (about 67 000 configurations in $C_s$).[10]

The calculated geometries and energies of the reactant, products, and transition state are shown in Figure 1 and Table I. The transition state has the $C_{2v}$ symmetry, and its reaction coordinate, the only normal coordinate with an imaginary frequency, shown by the arrows in Figure 1, consists mainly of the $H_2$ relative motion and the PPtP bending motion. It leads to the cis product. The PPtP angle is bent about 30° from that (180°) of the reactant. The HH bond is only 4% longer than that of the free $H_2$. The transition state is in an early stage of reaction, a reasonable finding for an exothermic reaction. In a kinetic study of another $H_2$ addition reaction $IrCl(CO)(PPh_3)_2 + H_2$, it has been suggested that the HH bond stretching is small at the transition state.[11]

The transition state yielding directly the trans product was not found. A "transition state", found with the $H_2$ axis kept perpendicular to the PPtP plane, belongs to the $C_{2v}$ symmetry and has two normal modes of imaginary frequency, one leading downhill to the trans product and the other through an $H_2$ rotation to the reaction path for the cis product. This is not surprising, because the trans addition to $ML_2$ with $d^{10}$ configuration is symmetry forbidden.[12] It is likely, therefore, that the reaction proceeds first via cis addition, which could be followed by the isomerization to the trans product through one of suggested paths[13] such as a five-coordinate complex involving a solvent molecule.

Our best calculation (CI + QC) gives a barrier height of 7 kcal/mol for the cis addition. The zero-point energy correction (ZPC) based on the RHF calculated force constants changes the effective barrier to 8 kcal/mol. Though these values should be taken to be only semiquantative, it is safe to say that the barrier for this model reaction is low, consistent with the experimental fact that oxidative addition reactions usually take place easily.

The geometries of the reactant and the products in the smaller basis set in Figure 1 compare favorably with those in the larger set as well as known experimental results of related compounds.[5] The energy difference between *cis*- and *trans*-$Pt(H)_2(PH_3)_2$ is within a few kilocalories per mole for all the methods in Table I and is certainly below the reliability of the present calculation. Most of the experimentally known diphosphine Pt(0) complexes have bulky phosphines as ligands.[1] Bulky phosphines probably will raise the barrier by destabilizing the transition state. The steric destabilization is probably even more serious in the cis product because of a smaller PPtP angle (104°). This may account for the reason why only trans products have been isolated, except for cis products of chelating phosphines where the isomerization path is obviously closed.[2]

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## Chemical Modification of Deoxyribonucleic Acids: A Direct Study by NMR Spectroscopy

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Chemical modification of biological macromolecules,[1-4] particularly polynucleotides,[1,5-12] is one of the promising approaches for studying the structure and function of biopolymers and bioactive substances.[13-15] It is evident that the success of this

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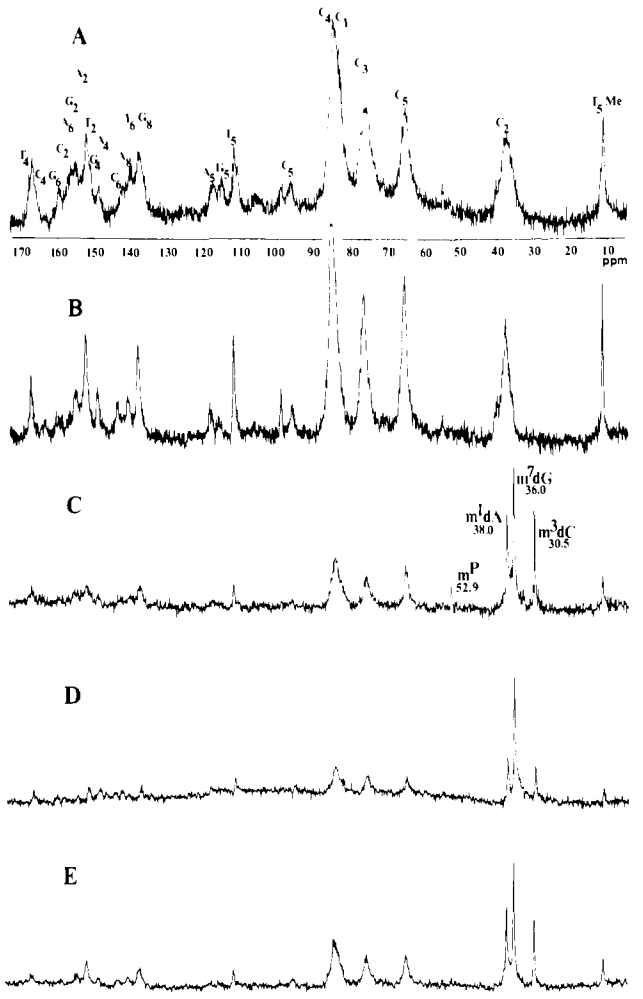


**Figure 1.** Proton-decoupled $^{13}C$ NMR spectra of DNA in 1.5 mL of $D_2O$ at pD 7.4 and 5 °C. The spectra were measured on Jeol PFT-100 at 25.15 MHz and a repetition time of 2 s (23-μs pulse) using 10-mm sample tubes. (A) Unmodified salmon sperm DNA (180 000 scans; 200 mg/1.5 mL). (B) Salmon sperm DNA reacted with MeMS for 12 h (212 000 scans; 200 mg/1.5 mL). (C) Salmon sperm DNA reacted with 90% $^{13}C$-enriched MeMS for 3 h (82 000 scans; 130 mg/1.5 mL). (D) Salmon testes DNA reacted with 90% $^{13}C$-enriched MeMS for 78 h (272 000 scans; 40 mg/1.5 mL). (E) Alkaline phosphatase digested salmon sperm DNA reacted with 90% $^{13}C$-enriched MeMS for 3 h. (214 000 scans; 75 mg/1.5 mL). Reference: tetramethylsilane. $m^3dC$, 3-methyldeoxycytidine; $m^7dG$, 7-methyldeoxyguanosine; $m^1dA$, 1-methyldeoxyadenosine; $m^P$, phosphomethyl group.

approach relies upon the accurate measurement of the reaction specificity sites of modification and product distribution. Radioisotope labeling has generally been used in detecting the degree of overall modification. However, in order to obtain specific information on a covalent interaction, it is necessary to carry out a series of systematic degradations followed by isolation, separation, and purification. This approach is not only tedious but also can often lead to erroneous conclusions due to secondary chemical alteration and/or decomposition. It is therefore important to develop more direct methods to determine the chemical modification of macromolecules. In this communication, we would like to report the application of NMR in the direct study of chemical modification of deoxyribonucleic acid.

Both salmon sperm DNA (ICN Nutritional Biochemicals) and salmon testes DNA (Sigma Chemical Co.) were purified by conventional enzymic digestions [bovine pancreatic ribonuclease A and *Streptomyces griseus* protease VI (Sigma Chemical Co.)],

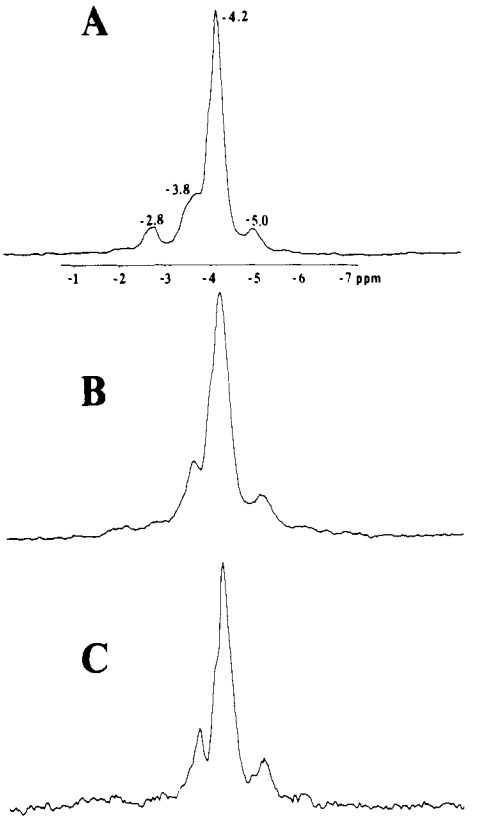


**Figure 2.** Proton-decoupled $^{31}P$ NMR spectra of salmon sperm DNA in 1.5 mL of $D_2O$ at pD 7.4 and 20 °C. The spectra were measured on Varian FT-80 at 32.2 MHz and a repetition time of 2 s (10-μs pulse) using 10-mm sample tubes. (A) Unmodified DNA (5000 scans; 60 mg/1.5 mL). (B) Alkaline phosphatase digested DNA [Salmon sperm DNA (1.2 mM) in 5 mL of 0.1 M Tris buffer pH 8.0 was treated with 90 units of *E. coli* alkaline phosphatase for 1 h at 37 °C] (8600 scans; 60 mg/1.5 mL). (C) Unmodified DNA reacted with 90% $^{13}C$-enriched MeMS for 12 h (8700 scans; 60 mg/1.5 mL). Reference: trimethyl phosphate.

phenol extraction, ethanol precipitation,[16,17] and extensive dialysis (Spectrapor 2 membrane, $M_r$ cutoff 14 000). The first modification was carried out by treating 202 mg of salmon sperm DNA with 50 μL (0.6 mmol) of methyl methanesulfonate (MeMS) (Aldrich Chemical Co.) in 6.47 mL of water at room temperature and pH 7.00 ± 0.01. The pH was controlled by a pH stat. After 3 h the reaction mixture was dialyzed, lyophilized, and subjected to NMR analysis.

The chemical shift assignments of unmodified DNA (Figure 1A) were primarily derived from comparing with those of corresponding monomers,[18,19] which are compatible with recently published data.[20] The $^{13}C$ natural abundance spectrum of the unmodified DNA and that of methylated DNA are shown in Figure 1. No methyl carbon signal is clearly observable in Figure 1B due to the low degree of reaction. Some of the methyl signals may even be obscured by the C-2′ signal of deoxyribose. The limited solubility and high molecular weight of DNA resulted in poor signal-to-noise ratio of the $^{13}C$ natural abundance signals. This difficulty can be overcome by using 90% $^{13}C$-enriched MeMS which could be readily prepared from the reaction of $^{13}C$-enriched methanol with methanesulfonic anhydride.[21] Since the natural

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abundance of $^{13}C$ nucleus is only 1.1%, any possible spectral interference from $^{13}C$ natural abundance signal of DNA, as shown in Figure 1B, can be greatly reduced (Figure 1C). Furthermore, this method also permits us to detect the modification at an early stage or reduce the amount of modifying agent, which may be critical since a biological event often takes place after exposure to a limited amount of active agent. Figure 1C distinctly displays four methyl carbon signals at 30.5, 36.0, 38.0 and 52.9 ppm, whose resonance designations as shown in Figure 1C can be determined on the basis of model studies of 31 different methylated nucleosides and nucleotides prepared in our laboratory. The product distribution can be determined from the integration curve.[12,22] The degree of modification may be calculated from the measurement of radioactivity by using [*methyl*-$^{14}C$]MeMS and [*methyl*-$^{13}C$]MeMS concurrently.

The observed line widths for salmon sperm DNA are approximately one-half of those for double-stranded calf thymus DNA of ~140 base pairs isolated from enzymatic degradation of chromatin.[20] The $^{13}C$ line widths and solubility in water indicates that this DNA is relatively small.[23] To study the tertiary structure effect on the chemical modification of DNA, we further modified the native salmon testes DNA[23] with [*methyl*-$^{13}C$]MeMS. Its $^{13}C$ spectrum is shown in Figure 1D. Comparison of Figure 1C,D indicates that the relative product distributions are quite different. The most remarkable difference is that the peak at 52.9 ppm corresponding to phosphomethyl signal is absent in the spectrum of alkylated salmon testes DNA. These results suggested that only the terminal phosphate group of polynucleotide can be significantly methylated. In order to test this suggestion, we treated 1.2 mm of salmon sperm DNA in 5 mL of 0.1 M Tris buffer (pH 8) with 90 units of *E. coli* alkaline phosphatase (Sigma Chemical Co.) for 1 h at 37 °C and then reacted it with [*methyl*-$^{13}C$]MeMS. The $^{13}C$ spectrum (Figure 1E) and $^{31}P$ NMR spectra[24] (Figure 2) unambiguously confirm this suggestion, since the enzyme treated DNA no longer has terminal phosphate groups, and indeed no phosphate alkylation is observed.

The approach outlined here provides a useful method for the direct study of the chemical modification of nucleic acids. Because this method avoids tedious and sometimes complicating degradative reactions of alkylated DNA, it should provide an important new approach to the determination of the sites of alkylation and mechanism of action of mutagens, carcinogens,[25-28] and anticancer agents.[29-31]

**Acknowledgment.** We gratefully acknowledge financial support from the National Cancer Institute (Grant number CA22987). We also acknowledge helpful discussions with Heinz G. Floss and technical assistance from John Kozlowski. $^{31}P$ NMR spectrum of methylated DNA was obtained from the Biomedical Magnetic Resonance Facility (National Institutes of Health, RR01077) at Purdue University.

(22) The product distributions are significantly different from previous ones determined by indirect methods (see review by: Singer, B. *Prog. Nucl. Acid Res. Mol. Biol.* **1975**, *14*, 219). However most results from other groups were obtained under different conditions (source of DNA, buffer solutions, pH values, temperature, ratio of alkylating agent to DNA). It may not be appropriate to make direct comparison and elaboration on their difference at this moment.

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| | salmon sperm DNA | salmon testes DNA |
|---|---|---|
| dA | 26.2 | 29.4 |
| dG | 20.3 | 22.0 |
| dT | 30.7 | 27.9 |
| dC | 22.8 | 20.6 |
| % double strandedness | 22 | 65 |
| $M_r$ | 92 000 | $7.4 \times 10^6$ |

(24) A detailed report on the $^{31}P$ NMR of modified nucleic acids will be published elsewhere. From Figure 2A,B, the most downfield peak at -2.8 ppm from trimethyl phosphate could be assigned to the terminal phosphate group. Methylation of this phosphomonoester caused it to overlap with one of the phosphodiester signals at -3.8 ppm (Figure 2C). (Davanloo, P.; Armitage, I. M.; Crothers, D. M., *Biopolymers* **1979**, *18*, 663-680). All signals from -3.8 to -5.0 ppm were ascribed to the $^{31}P$ resonance of random coil polynucleotide (Mariam, Y. H.; Wilson, W. D.; *Biochem. Biophys. Res. Commun.* **1979**, *88*, 861-866).

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## The Transition-Metal Nitro-Nitrosyl Redox Couple: Catalytic Oxidation of Olefins to Ketones

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Catalytic air oxidation is the method of choice for the industrial synthesis of a wide variety of chemicals. A new approach to this problem employs the transition-metal nitro-nitrosyl redox couple:

$$\text{M-NO}_2 + \text{L} \rightarrow \text{M-NO} + \text{L-O} \quad (1)$$

$$\text{M-NO} + {}^1/_2\text{O}_2 \rightarrow \text{M-NO}_2 \quad (2)$$

$$\text{L} + {}^1/_2\text{O}_2 \rightarrow \text{L-O}$$

While examples of reaction 1 have been known since at least 1962[1] and examples of reaction 2 since 1970,[2] a catalytic system that exhibits both reactions 1 and 2 was not reported until 1976.[3] The metal nitro-nitrosyl redox couple has been applied to the catalytic oxidation of CO to $CO_2$[3] and phosphines to phosphine oxides.[4] We report here a new nitro-nitrosyl redox couple based on the readily prepared complex bis(acetonitrile)chloronitropalladium(II) which catalytically air oxidizes olefins to ketones. Mechanistic evidence presented includes $^{18}O$ labeling data, spectroscopic data for intermediates, and the effect of olefin substituents. While this work was in progress, workers at Allied Chemical reported[5] that nitrosyltetraphenylporphyrincobalt is also a catalyst for the air oxidation of olefins to ketones; however, a Pd(II) cocatalyst was required and mechanistic data were limited.

*trans*-Bis(acetonitrile)dichloropalladium(II),[6] a common precursor for palladium(II)-olefin complexes,[6b,7] was treated with 1 or 2 mol of silver nitrite in acetonitrile to give quantitative yields

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