is outside the hydrogen-bonding region. We have now discovered a way in which coordination of a platinum(II) complex at the N(7) position may interfere with the hydrogen-bonding region and cause unusual base pairing.

We have been studying a series of complexes containing the *cis*-$Pt(NH_3)_2^{2+}$ unit attached to 9-ethylguanine (9-EtG) and 1-methylcytosine (1-MeC), crystallizing at various pHs. The compound crystallizing from water at pH 7 is $[Pt(NH_3)_2(9\text{-}EtG)(1\text{-}MeC)]^{2+}[Pt(NH_3)_2(9\text{-}EtG\text{-}H)(1\text{-}MeC)]^{+}(ClO_4)_3$, where 9-EtG--H is the deprotonated base. The compound was prepared by dissolving $[Pt(NH_3)_2(9\text{-}EtG)(1\text{-}MeC)]^{2+}(ClO_4)_2$ in water (solution pH 4.5) and titrating the solution with 0.2 N NaOH to pH ~8.4 under N_2. Evaporation gives the desired product in 65% yield. Crystals were obtained by heating the product in a little water to 75 °C and slowly cooling the resulting solution.[29] Crystal data for $C_{24}H_{43}Cl_3N_{20}O_{16}Pt_2$: M_r 1364.3; monoclinic; $C2/c$; $a = 23.16$ (8), $b = 11.971$ (3), $c = 16.140$ (4) Å; $\beta = 106.45$ (2)°; $Z = 4$; $d_{calcd} = 2.08$ g cm^{-3}. Intensity data were collected by using a Syntex P2$_1$ diffractometer with Mo Kα radiation. The platinum atom was located from a Patterson map, and the other nonhydrogen atoms were located by successive electron-density difference syntheses. Full-matrix least-squares refinement with anisotropic temperature factors for Pt and Cl converged to a conventional R value of 0.055 for 2536 reflections with $I > 3\sigma(I)$.

The molecular cation is shown in Figure 1. Bonding in the cations is normal, Pt being attached to N(7) of 9-EtG and N(3) of 1-MeC. The arrangement of ligand atoms about each platinum atom is essentially a square, and the Pt-N distances [Pt-Am(1), 2.06 (1); Pt-Am(2), 2.06 (1); Pt-N(3), 2.02 (1); Pt-N(7a), 2.04 (1) Å] and N-Pt-N angles [(Am(1)-Pt-Am(2), 88.8 (4); Am-(1)-Pt-N(7a), 90.2 (4); Am(2)-Pt-N(3), 90.4 (4); N(3)-Pt-N-(7a), 90.6 (4)°] are similar to those we have observed previously in other ammonia-base complexes of Pt(II).[21-23,30,31] Bond lengths and angles within 1-methylcytosine and 9-ethylguanine do not differ significantly from the average values listed by Voet and Rich.[32]

Significant features of the structure are the large dihedral angles between the planes of the bases and the square plane (N_2PtN_2-1-MeC, 68°; N_2PtN_2-9-EtG, 74°; 1-MeC-9-EtG, 79°). Because there are only four formula units in the unit cell, one perchlorate ion sits at a twofold axis and is disordered. The protonated and deprotonated cations are related by the twofold axis at $x = 0$, $z = 1/4$, and we assume that the hydrogen atom attached to N(1a) is disordered about this position. The interaction between the two cations is represented schematically in Figure 2. Hydrogen-bond distances are O(6a)-N(2a)′, 2.99 (2) Å; N(1a)-N(1a)′, 2.77 (2) Å. Thus, the coordination of Pt(II) at the N(7) position has shifted the pK of the N(1) position sufficiently to produce significant amounts of both protonated and deprotonated guanine at pH 7 and allow the G-G base pairing (Figure 2b), which is quite unlike that observed in poly(G) and poly(dG) helices,[33-35] guanosine and guanine gels,[36] or the Donohue structures.[37] The hydrogen bonding is very much like the Watson-Crick[38] hydrogen bonding between G-C pairs (Figure 2a). There are differences concerning the arrangement of the deoxyribose positions, and there is the difference in distance between these positions (13.11 Å in the G-G compound, 10.85 Å for a G-C pair[39]) which would make packing a G-G pair within a molecule very difficult.

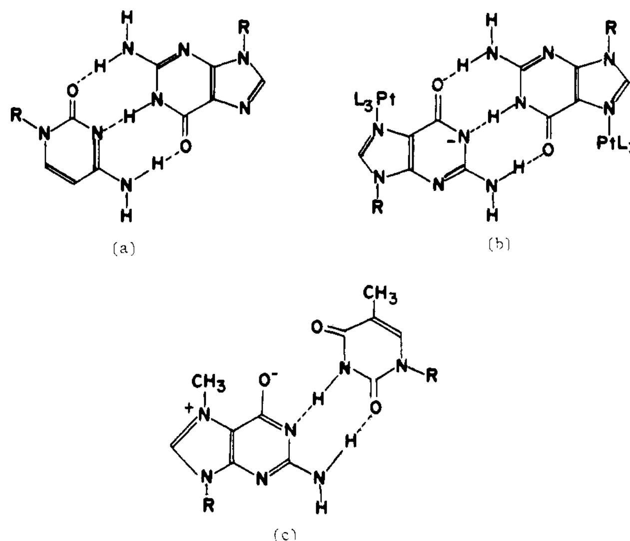

Figure 2. A comparison of the (a) Watson-Crick G-C pair, (b) the platinated G-G pair, and (c) the methylated G-T pair.

There is, however, an alternate way that base mispairing in DNA could be induced. The guanine in the deprotonated cation looks very much like the N(7) methylated guanosine which can bond in the manner shown in Figure 2c to thymine to give a G-T pair.[40] Thus, we have a very simple model of how a pK shift caused by platinum coordination at N(7) of guanine might give rise to a G-T mispairing in DNA. It will be necessary to do further work to see whether other cis or trans complexes can induce the same effect.

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Cleavage of DNA by Coordination Complexes. Superoxide Formation in the Oxidation of 1,10-Phenanthroline-Cuprous Complexes by Oxygen—Relevance to DNA-Cleavage Reaction

Sir:

The 1,10-phenanthroline-cuprous complex, $(OP)_2Cu^+$, cleaves DNA in an oxygen-dependent reaction.[1] Cleavage of 10 µg/mL of poly(dA-T) by micromolar levels of coordination complex can be readily measured within 1 min because the products of the degradation are effective inhibitors of *E. coli* DNA polymerase I. The blockage of the reaction by catalase and the failure of the oxidatively stable 2,9-dimethyl-1,10-phenanthroline-cuprous complex[2] to degrade DNA indicate that intermediates formed

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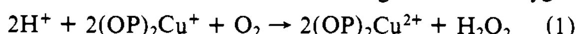
during the oxidation of $(OP)_2Cu^+$ may participate in the chemistry of the cleavage of DNA by the coordination complex.

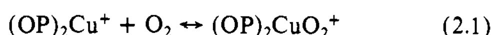
However, the reaction mechanism for the oxidation of $(OP)_2Cu^+$ by molecular oxygen in aqueous solution has not yet been determined. Mechanistic schemes that have been proposed to account for the oxidation of the cuprous complexes to yield H_2O_2 (eq 1) include a two-electron transfer involving a binuclear oxygen



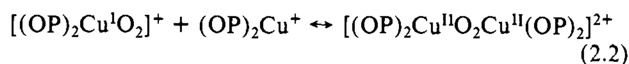
$$2H^+ + 2(OP)_2Cu^+ + O_2 \rightarrow 2(OP)_2Cu^{2+} + H_2O_2 \quad (1)$$

adduct (Scheme I) or a stepwise reduction of O_2 involving superoxide as an intermediate (Scheme II).[3-5] In nitromethane

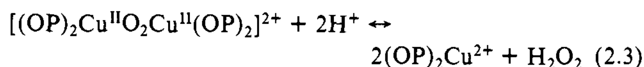
Scheme I



$$(OP)_2Cu^+ + O_2 \leftrightarrow (OP)_2CuO_2^+ \quad (2.1)$$

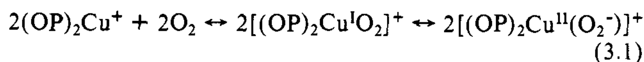


$$[(OP)_2Cu^IO_2]^+ + (OP)_2Cu^+ \leftrightarrow [(OP)_2Cu^{II}O_2Cu^{II}(OP)_2]^{2+} \quad (2.2)$$



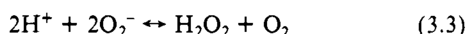
$$[(OP)_2Cu^{II}O_2Cu^{II}(OP)_2]^{2+} + 2H^+ \leftrightarrow 2(OP)_2Cu^{2+} + H_2O_2 \quad (2.3)$$

Scheme II



$$2(OP)_2Cu^+ + 2O_2 \leftrightarrow 2[(OP)_2Cu^IO_2]^+ \leftrightarrow 2[(OP)_2Cu^{II}(O_2^-)]^+ \quad (3.1)$$

$$2[(OP)_2Cu^{II}O_2^-]^+ \leftrightarrow 2(OP)_2Cu^{2+} + 2O_2^- \quad (3.2)$$

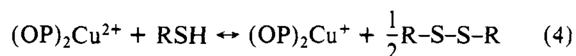


$$2H^+ + 2O_2^- \leftrightarrow H_2O_2 + O_2 \quad (3.3)$$

solution, the rate law for the oxidation of $(OP)_2Cu^+$ exhibits a second-order dependence on the concentration of the coordination complex, suggestive of Scheme I.[6] However, the relevance of these results to aqueous solution is not certain since (a) only a first-order dependence on the complex is observed in aqueous acetonitrile[5] and (b) the redox potential for the O_2/O_2^- potential is more positive in water than in aprotic solvents.[7-9] An important finding favoring Scheme II is that the reduction of $(OP)_2Cu^{2+}$ by superoxide, the microscopic reverse of the oxidation of $(OP)_2Cu^+$ via superoxide anion, has been demonstrated in dimethyl sulfoxide.[9] It has been further noted that the position of the equilibrium summarized by eq 3.1 and 3.2 should be sensitive to the availability of protons.[9]

In this communication, we present direct evidence for the formation of superoxide anion in the oxidation of $(OP)_2Cu^+$ in aqueous solution and demonstrate that Scheme II is an important pathway for the oxidation of $(OP)_2Cu^+$ in water, accounting for a minimum of 45% of the electron transfer at pH 7.0. However, diffusible superoxide does not react directly with the DNA to cause cleavage. If generated by xanthine and xanthine oxidase, superoxide potentiates the cleavage reaction by $(OP)_2Cu^{2+}$ because it increases the concentration of $(OP)_2Cu^+$ and hydrogen peroxide, the reactants which are probably directly responsible for the cleavage.

In order to approximate the conditions of the DNA cleavage reaction, the oxidation of $(OP)_2Cu^+$, generated by the in situ reduction of the cupric complex by thiol (eq 4), was studied in



$$(OP)_2Cu^{2+} + RSH \leftrightarrow (OP)_2Cu^+ + \frac{1}{2}R\text{-}S\text{-}S\text{-}R \quad (4)$$

the presence of nitro blue tetrazolium (NBT).[10] The reduced form of the dye can be readily measured because it absorbs at 560 nm with an extinction coefficient of 22 400 M^{-1} cm^{-1} while the oxidized NBT absorbs minimally at this wavelength. Addition of the thiol 3-mercaptopropionic acid to an aqueous aerobic solution (O_2, 2×10^{-4} M) of $(OP)_2Cu^{2+}$ results in an easily measurable absorption increase due to the reduction of NBT (Figure 1). Since the reduction of NBT is strongly inhibited by

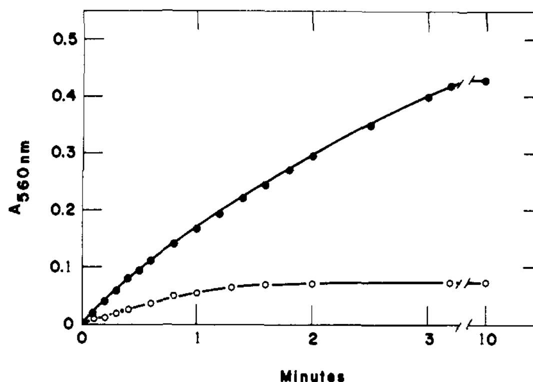


Figure 1. Reduction of nitro blue tetrazolium (NBT) under aerobic conditions by thiol and $(OP)_2Cu^{2+}$. Cupric acetate was added to initiate NBT reduction. The reaction mixture contained the following components: NBT, 6.1×10^{-3} M; 3-mercaptopropionic acid, 10^{-4} M; O_2, 2×10^{-4} M; OP, 4×10^{-6} M; cupric acetate, 1×10^{-6} M (●). Superoxide dismutase (800 units) added to an otherwise identical reaction mixture (○). NBT reduction was monitored continuously at 560 nm by using a Gilford Spectrophotometer Model 250, equipped with an automatic sample changer (ϵ_{560} of reduced NBT = 22 400 cm^{-1} M^{-1}, 21 °C).

bovine erythrocyte superoxide dismutase, the formation of superoxide during the oxidation of $(OP)_2Cu^+$ is strongly implied (Figure 1). The possibility that superoxide dismutase blocks NBT reduction by scavenging cupric ion and decreasing the concentration of the copper complexes of 1,10-phenanthroline has been excluded by comparing the enzyme's inhibition of NBT reduction to its effect on the rate of thiol oxidation (eq 4), measured with 5,5'-dithiobis(2-nitrobenzoic acid).[11-13] Since thiol oxidation is only inhibited 10% by superoxide dismutase under conditions where NBT reduction is more than 70% blocked (e.g., Figure 1), the catalytic activity of the enzyme and not its metal ion binding properties is responsible for the observed effect on NBT reduction. The failure to detect more than trace levels of NBT reduction under anaerobic conditions is further evidence for superoxide production.

The yield of reduced NBT when thiol is limiting is related to the extent that the net oxidation of $(OP)_2Cu^+$ by molecular oxygen proceeds via superoxide formation. In the experiment summarized in Figure 1, 22% of the electrons transferred from the thiol can be trapped with 6.1 mM NBT. At NBT concentrations of 20 mM, 45% of the reducing equivalents can be captured by the superoxide trap. Scheme II, involving the formation of diffusible superoxide, must represent a major reaction pathway. It is not possible to conclude that it is the exclusive pathway on the basis of the data presently available.

When ascorbic acid replaces mercaptopropionic acid as the reducing agent, NBT reduction is also observed. Since the reduction of NBT by ascorbate mediated by $(OP)_2Cu^+$ is also inhibited by superoxide dismutase, superoxide production is not dependent on the chemical structure of the electron donor and must be generated in the common step, the reoxidation of $(OP)_2Cu^+$.

Further evidence for the latter conclusion is that superoxide has also been detected in the reoxidation of $(OP)_2Cu^+$ prepared as the crystalline perchlorate salt by refluxing $(OP)_2Cu^{2+}$ with hydroxylamine in 20% ethanol with 0.8% ammonia.[14] Addition of aliquots of acetonitrile or dimethyl sulfoxide solutions of this salt to air-saturated Tris–acetate buffer (pH 7.0) leads to the reoxidation of this complex within 2 s, as measured by the loss of its characteristic absorption maximum at 430 nm. If NBT is present in the buffer, the absorbance increase at 560 nm characteristic of the reduction of the dye is observed. Tetranitro-

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Table I. Effect of Xanthine and Xanthine Oxidase on DNA Cleavage Measured by Inhibition of *E. Coli* Polymerase I

incubation conditions[a]	% activity[b]
control	100
OP (12.5 μM), Cu^{2+} (1 μM)	103
xanthine (10^{-4} M), xanthine oxidase (6.7×10^{-3} units/mL), Cu^{2+} (1 μM)	80
xanthine (10^{-4} M), xanthine oxidase (6.7×10^{-3} units/mL), Cu^{2+} (1 μM), catalase (50 μg/mL)	97
xanthine (10^{-4} M), xanthine oxidase (6.7×10^{-3} units/mL), OP (12.5 μM), Cu^{2+} (1 μM)	4
xanthine (10^{-4} M), xanthine oxidase (6.7×10^{-3} units/mL), OP (12.5 μM), Cu^{2+} (1 μM), catalase (50 μg/mL)	84

[a] All incubation mixtures contained [^{3}H]TTP (50 μM), dATP (50 μM), and poly(dA-T) (10 μg/mL). Incubation time, 3 min. [b] Percent activity, 100 s after addition of *E. coli* polymerase I (see ref 1 for details).

methane, another effective superoxide trap, was used to demonstrate the presence of superoxide in the stoichiometric oxidation of the crystalline perchlorate salt of $(OP)_2Cu^+$.[15] Tetranitromethane could not be used in the in situ reduction and reoxidation of $(OP)_2Cu^+$ because it reacts with free sulfhydryl groups. $(OP)_2Cu^+$, prepared by the addition of OP to an acetonitrile solution of $Cu(CH_3CN)_4ClO_4$,[16] is indistinguishable in its reactivity to the crystalline cuprous complex described above. If thiol or ascorbic acid is added to the aerobic aqueous solution used to oxidize either of these complexes, the steady-state reduction of NBT is observed.

The cupric complexes of 2,2′,2″-terpyridine and bipyridine produce diffusible superoxide at rates comparable to $(OP)_2Cu^{2+}$ in the presence of thiol and O_2. Since the 1,10-phenanthroline–copper complex is unique in causing inhibition of DNA polymerase by cleaving DNA,[17,18] diffusible superoxide cannot be directly responsible for the cleavage reaction. This result is supported by the failure of superoxide generated by xanthine and xanthine oxidase to cleave DNA and inhibit the enzyme (Table I). However, xanthine and xanthine oxidase can potentiate the cleavage of DNA by $(OP)_2Cu^{2+}$ in a reaction that is blocked by catalase (Table I). Since the DNA cleavage reaction and its associated *E. coli* polymerase I inhibition are blocked by 2,9-dimethyl-1,10-phenanthroline, a cuprous ion specific chelator,[17,18] the potentiation by superoxide can be readily explained if $(OP)_2Cu^+$ and H_2O_2 are the essential reactants for the DNA cleavage reaction. Superoxide can enhance the concentration of $(OP)_2Cu^+$ by reduction of $(OP)_2Cu^{2+}$ (eq 3.1 and 3.2)[9] and generate hydrogen peroxide by its spontaneous dismutation (eq 3.3). Like any other reducing agent, superoxide enhances the DNA cleavage reaction by increasing the concentrations of $(OP)_2Cu^+$ and hydrogen peroxide.

In this report, we have established for the first time that the oxidation of the cuprous complex of 1,10-phenanthroline and related ligands by oxygen proceeds via a superoxide intermediate. However, it is clear that diffusible superoxide is not directly involved in the DNA cleavage reaction caused by $(OP)_2Cu^{2+}$ and thiol under aerobic conditions. The ability of superoxide generators such as xanthine and xanthine oxidase to substitute for thiol in the cleavage reaction can be fully explained in terms of $(OP)_2Cu^+$ and hydrogen peroxide being essential reactants for the breakdown of the DNA. Studies in progress have confirmed this conclusion by showing that addition of hydrogen peroxide to an anerobic solution of $(OP)_2Cu^+$ causes the cleavage of DNA within 1 min.

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The exclusive reactivity of the 1,10-phenanthroline complex is striking. It may relate to its ability to intercalate into DNA in a unique orientation. Once bound to the polynucleotide, it may react directly with hydrogen peroxide to yield hydroxyl radicals via chemistry analogous to that of Fenton's reagent,[19] or it may facilitate the nucleophilic attack of hydrogen peroxide on the phosphodiester bond.

In passing, it is interesting to note that $(OP)_2Cu^{2+}$ is widely used to cross-link proteins via disulfide linkages.[20] Superoxide must be produced during this reaction and may lead to potential complexities in interpretation, especially when it is formed in a hydrophobic milieu where it is a potent nucleophile.[21]

Acknowledgments. This research was supported by USPHS Grant 21199. We thank Professor J. S. Valentine of the Department of Chemistry for valuable conversations and A. Mahmoudi for carrying out preliminary experiments.

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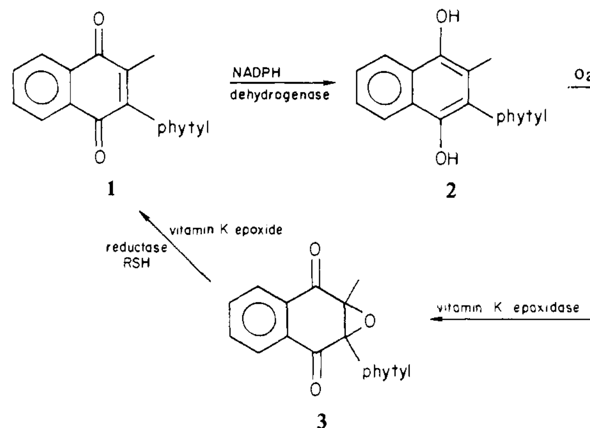
A Model for a Molecular Mechanism of Anticoagulant Activity of 3-Substituted 4-Hydroxycoumarins

Sir:

Vitamin K (**1**), a fat-soluble vitamin essential for the coagulation of blood, has been shown to be required for the biosynthesis of active prothrombin and other plasma clotting factors.[1] It has



been established that the active form of the vitamin is the reduced (hydroquinone) form[2] (**2**) and that an O_2-dependent epoxidase catalyzes the conversion of **2** to vitamin K 2,3-epoxide (**3**).[3] The epoxidase activity has been linked with the activation of prothrombin and the coagulation of blood.[4] It has been suggested that vitamin K epoxide is biosynthesized as a means of intracellular storage of an inactive form of the vitamin.[5] Because of the

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