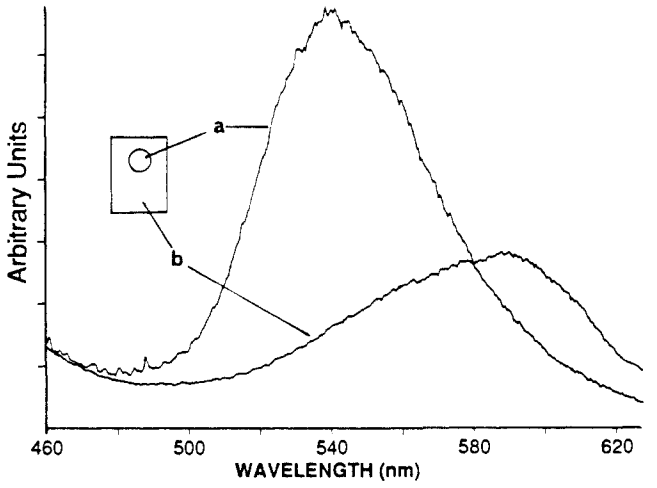

Figure 1. Emission from Cu^+-doped β''-alumina at 10 K: (a) dimer emission from the "written" spot and (b) emission from the region cooled in the dark.

concentrations were determined by chemical analysis (ICAP, Coors Laboratories). In a typical sample, the copper concentration was approximately 5×10^{18} ions/cm^3 (copper:sodium $\cong$ 1:1000). Luminescence spectra were obtained, and optical writing was achieved with the 351-nm line from an argon ion laser. The output power was approximately 30 mW for all of the experiments.

The doped crystals show a green luminescence ($\lambda_{max} \sim 540$ nm) at room temperature.[1] If the crystal is cooled to 10 K in the dark, the green emission is reduced in intensity. In addition, a red emission peak ($\lambda_{max} \sim 590$ nm) and two blue emission peaks ($\lambda_{max} \sim 410,440$ nm) are observed. If a spot is irradiated as the crystal is cooled, the intensity of the green emission from the spot increases. The spectrum from the spot is similar to the room temperature spectrum ($\lambda_{max} \sim 540$ nm). This spectral and spatial selectivity at 540 nm (Figure 1) constitutes the basis for the read/write characteristics of Cu^+-doped β''-alumina.

Optical writing was achieved by focussing the laser on small regions of the crystals. Away from the spot at which the laser had been focussed, the observed spectrum was similar to that obtained from samples cooled in the dark. Thus, the sample "remembers" where the laser was focussed. If the excitation is defocussed to cover the entire crystal, there is a spot of bright green emission on a less intense field. The process of irradiating a specific region during cooling and then detecting the irradiated region constitutes a write/read operation. Other experiments have indicated that it is not necessary to irradiated while the sample is cooling. It is sufficient to focus the laser on a sample held at temperatures less than 77 K to produce the changes in the spectrum. However, the best contrast between the written spot and the background is achieved if the sample is irradiated during cooling.

The changes are reversible. If the sample is warmed to room temperature, the green emission is again observed uniformly throughout the crystal. Thus, the memory can be "erased" and "rewritten".

The blue and green emissions are assigned to individual Cu^+ ions and to dimers, respectively. The blue emissions seen at low temperature are similar to the emissions of Cu^+ in alkali halide hosts.[5] Therefore, we assign these emissions to the $d^9s^1 \rightarrow d^{10}$ transition of the copper ion in the fields of oxide ions in the conduction plane. However, the green emission and its dependence on irradiation cannot be explained solely by emission from isolated copper ions. We assign this emission to copper pairs in the conduction plane. A similar emission was observed from Cu^+ in $SrCl_2$ which was attributed to Cu^+ pairs.[6]

The stability of d^{10}-d^{10} dimers has been the subject of several investigations.[7] Excitation of the dimer removes an electron from an antibonding orbital and populates a bonding orbital. This gives the dimers increased stability under UV irradiation. The formation of copper and silver dimers under UV irradiation in inert gas matrices has been reported by Ozin and co-workers.[8] Only wavelengths absorbed by the metal atoms were effective in inducing the formation of dimers. We find the same result here. Wavelengths longer than 351 nm do not produce the changes in the spectrum.

The mechanism of copper pair formation is associated with the ion conducting nature of the host lattice. At room temperature, the copper and sodium ions are mobile in the conduction plane. The measured conductivity at room temperature of sodium β''-alumina implies a hopping rate of $\sim 10^9$ s^{-1}. At this rate, the probability of a Cu^+ ion encountering another is high even at low doping concentrations. Once a dimer is formed, it may remain for a time before being forced apart (for example, by the action of the mobile Na^+ ions). The monomers do not emit at room temperature, and a uniform green emission is observed. As the crystal is cooled without excitation, the ions settle into the most stable sites. Emission is seen from all of the copper-containing species but primarily from the more prevalent Cu^+ monomers. If the sample is irradiated while it is cooled, the dimers that form remain together and are trapped as the temperature is lowered. This results in a higher concentration of dimers within the irradiated area and a visible difference in the emissions from the different parts of the crystals.

The novel ion transport properties of β'' alumina provide the opportunity to create materials in which the luminescent species are mobile. The photoaggregation of the Cu^+ dimers and the spectroscopic contrast between the monomers and dimers is the basis for the optical write/read characteristics of the material.

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Sequence-Specific Scission of RNA by 1,10-Phenanthroline–Copper Linked to Deoxyoligonucleotides

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Site-specific scission of DNA has been achieved by linking carrier ligands such as polynucleotides and protein to the oxidative nuclease activity of 1,10-phenanthroline–copper.[1,2] On the basis of product analysis, the following reaction pathway (see Scheme I) has been proposed for the DNase activity of the oxidative species formed by the DNA-bound 2:1 1,10-phenanthroline–cuprous complex and its coreactant hydrogen peroxide.[3-6]

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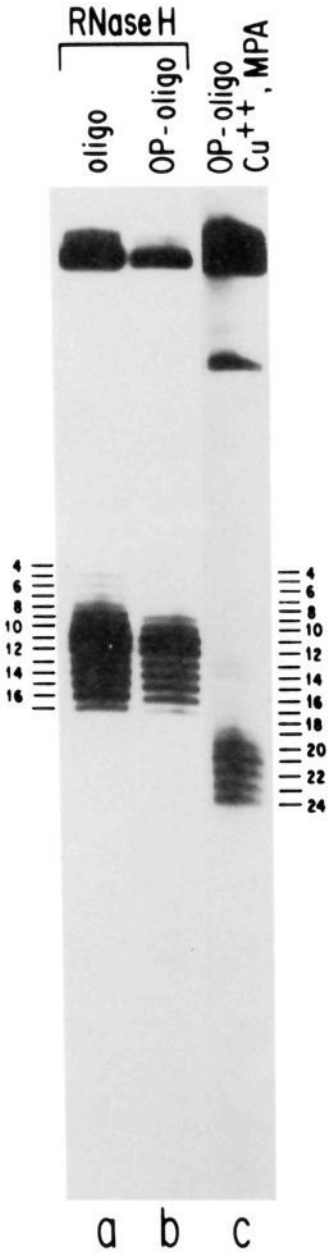


Figure 1. Digestion patterns of labeled lac RNA (positions −18 to +63): lane A, RNase H digestion of RNA directed by unsubstituted 21-mer of I; lane B, RNase H digestion of RNA directed by I; lane C, scission of RNA by I after hydridization and the addition of 3-mercaptopropionic acid and cupric ion under aerobic conditions. Number 1 corresponds to normal start site of lac mRNA transcription.

Similar reagents for RNA's would be useful, but their development requires that the oxidative scission of the phosphodiester backbone of RNA's be at least as efficient as that observed with DNA. Since the 2′ hydroxyl inhibits the acid-catalyzed depurination of RNA relative to DNA by a factor of 750,[7] the equivalent reactivity of RNA and DNA to the nucleolytic activity of 1,10-phenanthroline–copper cannot be assumed. The ribose moiety must not only be susceptible to oxidative attack but it must also undergo the elimination reactions essential for strand scission. Bleomycin, which effectively degrades DNA by an oxidative pathway, does not cleave RNA.[8]

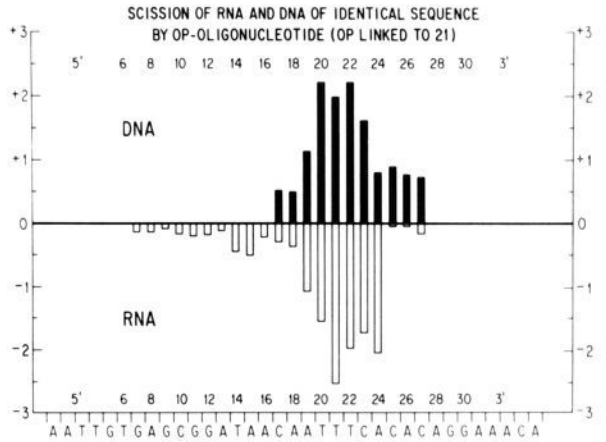
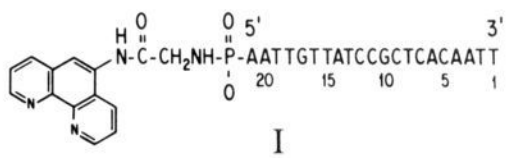


Figure 2. Comparison of the scission of RNA and DNA of identical sequence by I. Conditions are reported in footnote 12. Parallel reaction conditions for scission of DNA were used.[1]

In order to test the intrinsic reactivity of RNA and DNA to targeted scission by 1,10-phenanthrolinecopper, the OP-oligonucleotide (I), whose sequence corresponds to position +1 to +21



I

of the coding strand of the lac operon (+1 corresponds to the start of lac m-RNA) was used to cut an RNA substrate that extended from −18 to +63 and a DNA substrate extending from −124 to +63 (noncoding strand).[10] This experimental system ensures that kinetically important intermediate complexes, intrinsic to targeted chemical nuclease activities, will have comparable stabilities. Any difference in reactivity could therefore be attributable to the efficiency of the cleavage chemistry.

Since the 21 residue long oligonucleotide linked, or unlinked, to OP directed scission of RNA by RNase H[11] after heat denaturation at 65 °C and annealing at 0 °C (cf. lanes a and b, Figure 1), the 1,10-phenanthroline moiety at the 5′ end of the oligonucleotide did not interfere with hybridization. Hydrolysis is observed from positions +17 to +5 suggesting that the ends of the 21-mer may not be involved in Watson–Crick base pairing. To activate the cleavage chemistry, cupric ion and 3-mercaptopropionic acid are added, and the reaction mixture is incubated for 2 h at 37 °C.[12] The products of the reaction were analyzed on a sequencing gel; the major sites of cutting using I extend from sequence positions 19–24 (Figure 1). No cutting was observed in a control reaction in which the OP-oligonucleotide was replaced with an equimolar mixture of free 1,10-phenanthroline, cupric ion, and an underivatized oligonucleotide.

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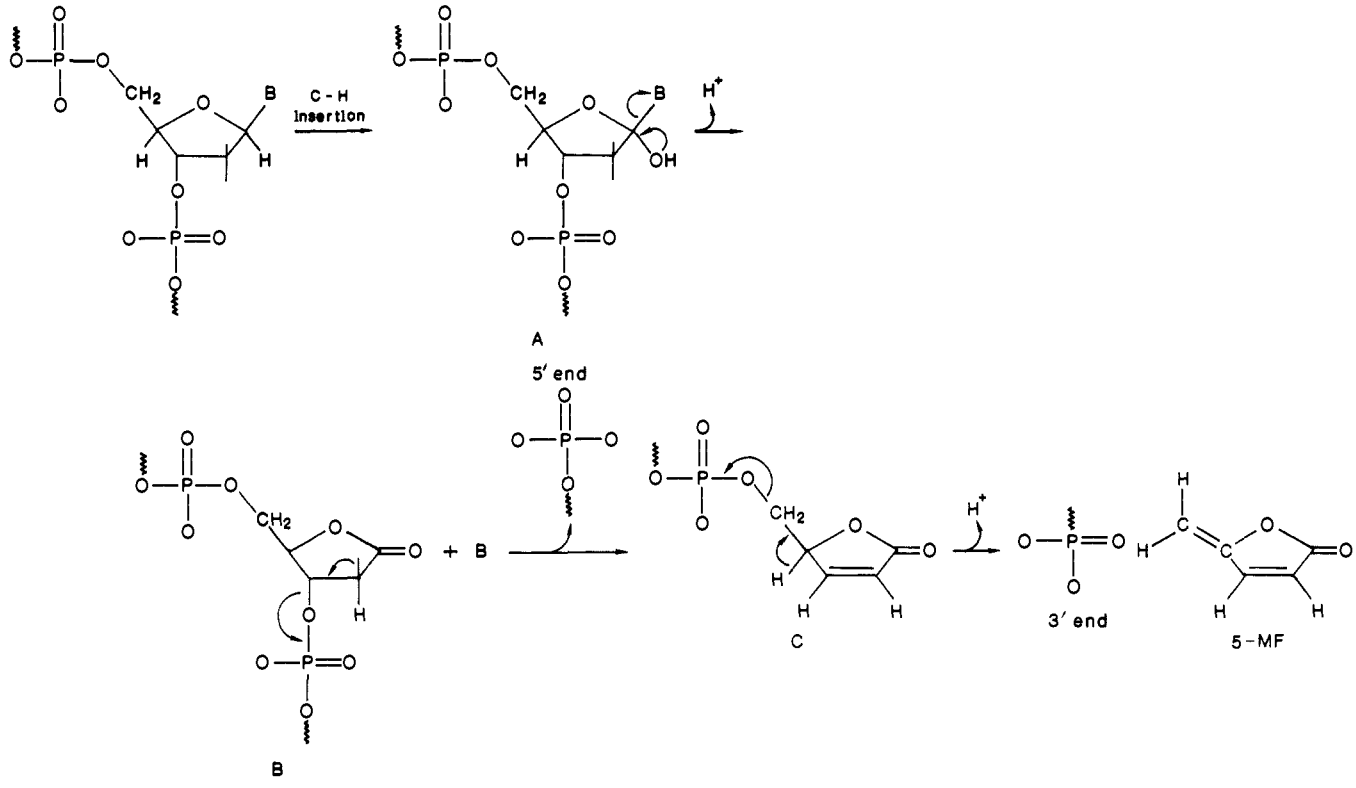
(12) Cleavage reaction of lac RNA. A 10-μL solution of lac RNA (0.25 μM) and I (10 μM) in 50 mM Tris pH 8.0/50 mM NaCl was heated at 65 °C for 3 min, quickly chilled on dry ice for 1 min, and then allowed to thaw on ice. After warming up to room temperature, the cleavage was initiated by adding 1 μL of $CuSO_4$ (20 μM) and 1 μL of MPA (58 mM) and incubated at 37 °C for 2 h. After quenching with 1 μL of 2,9-dimethyl-1,10-phenanthroline (28 mM), products were ethanol precipitated by adding 1.2 μL of 3 M NaOAc and 2.5 vol of absolute ethanol.

Scheme I



The product distribution from the 3′ labeled RNA strand is compared to that from the 3′ labeled noncoding DNA strand of the Eco R1 restriction fragment by using I as the nucleolytic agent (Figure 2). In both cases, the major cutting sites are clustered between positions 19–24. This interval of predominant cutting sites 2–3 nucleotides in both directions from the tethered 1,10-phenanthroline can be due to the diffusibility of the oxidative species or the flexibility of the 1,10-phenanthroline linked terminal deoxyadenosine that can be inferred from the RNase H hybridization studies. The kinetics of the cutting reaction are similar with both RNA and DNA. After incubation for 2 h at 37 °C, approximately 20% of the parent band is converted to one of the oligonucleotide products.

Primer extension assays were also used to monitor the reaction of I with RNA.[13] These assays reflect phosphodiester bond scission as well as any oxidative damage that may block polymerization. Since the pattern of products obtained were similar to those using 3′ labeled RNA, there is no evidence for reaction that does not lead to strand scission. In previous studies of the DNase activity of 1,10-phenanthroline–copper ion, no reaction without strand scission has ever been observed.[14,15]

The similarity in the digestion patterns suggests that the phosphodiester backbones of RNA and DNA are comparably reactive to the chemical nuclease activity of 1,10-phenanthroline–copper. The extension of these findings to other oxidative nucleolytic activities, e.g., ferrous-EDTA and iron porphyrins,[16-20] will require direct experimental tests in view of bleomycin's inability to nick RNA.

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Regio- and Stereoselective Reduction of N(21),N(22)-Bridged Porphyrin Hydroperchlorates to Stable 5*H*-Phlorins

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Hydroporphyrins with saturated meso carbon(s) are important in the redox chemistry and the biosynthesis of porphyrin.[1] Phlorins, 5*H*,22*H*-dihydroporphyrins, are usually so air-sensitive that only a few have been fully characterized so far[2] including those with the steric crowding of peripheral substituents which is relieved upon hybridization change from sp^2 to sp^3 of the meso carbon as shown in the Woodward's approach to chlorophyll *a*.[3]

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