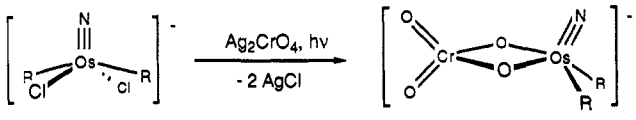
reactions proceeded only in the light. When the syntheses were attempted in an opaque vessel, no product was obtained. The IR and NMR spectra of these new chromate complexes suggests a similar structure with cis, bidentate chromate ligands. The IR bands associated with the chromate group in 1 and 2 are similar to those of $[Co(NH_3)_4(CrO_4)][NO_3]$, in which there is bidentate coordination of the ligand.[11]



1, R = CH_2SiMe_3; 2, R = Me

These osmium and ruthenium chromate complexes are quite thermally stable. Heating them in the solid state or in solution to 100 °C causes no decomposition. They are also stable to air and water. Even though these species are anionic, the presence of the alkyl groups and large organic counterion makes them soluble in a variety of organic solvents including toluene and diethyl ether.

The osmium chromate complex, *cis*-$[NBu^n_4][Os(N)(CH_2SiMe_3)_2(CrO_4)]$, catalytically oxidizes a variety of alcohols with high selectivity. Primary alcohols are oxidized exclusively to aldehydes and secondary alcohols to ketones. No carboxylic acids or esters could be detected in the reaction mixtures by gas chromatography or ^1H NMR. The reaction requires molecular oxygen, which is reduced to water.

In a typical reaction, a magnetically stirred mixture of *cis*-$[NBu^n_4][Os(N)(CH_2SiMe_3)_2(CrO_4)]$ (8 mg, 0.01 mmol, 5 mol%), alcohol (0.2 mmol), anisole (0.12 mmol, internal standard), and acetonitrile (2.0 mL) was heated to 70 °C for 72 h under air. Conversion of alcohol to aldehyde or ketone, as determined by gas chromatography and ^1H NMR, ranged from 57% to 9% depending on the nature of the alcohol. Similar results were obtained when the reactions were performed in a closed system under O_2, either in a pressure bottle or in a sealed NMR tube. No reaction occurred in the absence of the catalyst.

The rate of oxidation and yield of product depended on the nature of the substrate and reaction conditions. Oxidation of benzylic and allylic alcohols proceeded more rapidly than the oxidation of primary alcohols, and primary alcohols were oxidized more rapidly than secondary alcohols. Tertiary alcohols were not oxidized. In contrast, potassium chromate in aqueous acidic acid solution oxidizes hindered alcohols faster than less hindered alcohols. In the oxidation of benzyl alcohol to benzaldehyde, the rate and yield of the reaction increased when the reaction was run under oxygen (30 psi) or in air in the presence of cupric acetate.[12,13]

The less bulky osmium chromate complex, **2**, was more active for alcohol oxidation. Benzyl alcohol was oxidized in air with 79% conversion to benzaldehyde in 12 h. Again, the aldehyde was the only product of the reaction.[14]

The osmium chromate complex, **1**, is chemoselective for the oxidation of hydroxy groups. Neither triphenylphosphine nor olefins (cyclohexene, styrene) reacted with the osmium chromate complex under alcohol oxidation conditions. No oxidation of the double bond occurred in the catalytic oxidation of 2-cyclohexenol to 2-cyclohexanone. No reaction with carbon monoxide or pyridine was observed.

In all of the reactions with alcohols performed in air, **1** was eventually converted to a yellow-green species which has not yet been fully characterized. This product was not active for alcohol oxidation. Even though the osmium chromate complex is stable to water at room temperature, it may be decomposing under the reaction conditions with water formed as a byproduct.

The osmium–chromate complexes have a number of advantages over currently employed Cr(VI) complexes in the oxidation of alcohols. They are thermally stable and soluble in organic solvents. They are highly selective, giving only aldehyde in the oxidation of primary alcohols. The chemoselectivity allows their use in the presence of triphenylphosphine, olefins, amines, and ethers. They are unique with respect to other chromium(VI) reagents in their ability to activate molecular oxygen in the catalytic oxidation of alcohols.[15] The reaction is slow, however, and deactivation of the catalysts with the water produced may be occurring over time. Since ruthenium complexes are generally more powerful oxidants than the corresponding osmium species,[16] and ruthenium complexes oxidize alcohols,[17] we are now investigating the reactivity of the ruthenium–chromate complexes, $[Ru(N)R_2(CrO_4)]^-$, in alcohol oxidations.

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Supplementary Material Available: Experimental procedures and physical data for the new compounds (1) *cis*-$[NBu^n_4][Os(N)(CH_2SiMe_3)_2(CrO_4)]$, (2) *cis*-$[PPh_4][Os(N)(CH_3)_2(CrO_4)]$, (3) *cis*-$[NBu^n_4][Ru(N)(CH_2SiMe_3)_2(CrO_4)]$, and (4) *cis*-$[PPh_4][Ru(N)(CH_3)_2(CrO_4)]$ (2 pages). Ordering information is given on any current masthead page.

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(13) No catalytic oxidation occurred in the absence of the osmium chromate complex. A mixture of potassium chromate and cupric acetate converted a very small amount of benzyl alcohol (2.7% conversion, 0.51 TO) to oxidation products.

(14) Concentrations of reactants were the same as above: 0.01 mmol catalyst, 0.2 mmol benzyl alcohol, in 2.0 mL of acetonitrile.

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A Hybrid Sequence-Selective Ribonuclease S

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Chemists and biologists are focusing considerable effort on the design of selective catalysts for the synthesis and modification of complex molecules. One approach involves modifying the specificity of existing enzymes either by altering active site residues via site-directed mutagenesis or by selectively introducing new binding domains into enzymes. We recently applied this latter stategy to the construction of a hybrid enzyme capable of site-selectively hydrolyzing single-stranded DNA and RNA.[1,2] We now report the design of a second hybrid enzyme based on this strategy. A sequence-selective ribonuclease has been generated by selectively fusing an oligodeoxyribonucleotide binding site of defined sequence to the relatively nonspecific enzyme bovine pancreatic ribonuclease A.

Ribonuclease A (RNase A) is a well-characterized stable enzyme, 124 amino acids in length, that hydrolyzes the phosphodiester bonds of single-stranded RNA preferentially to the 3′ side

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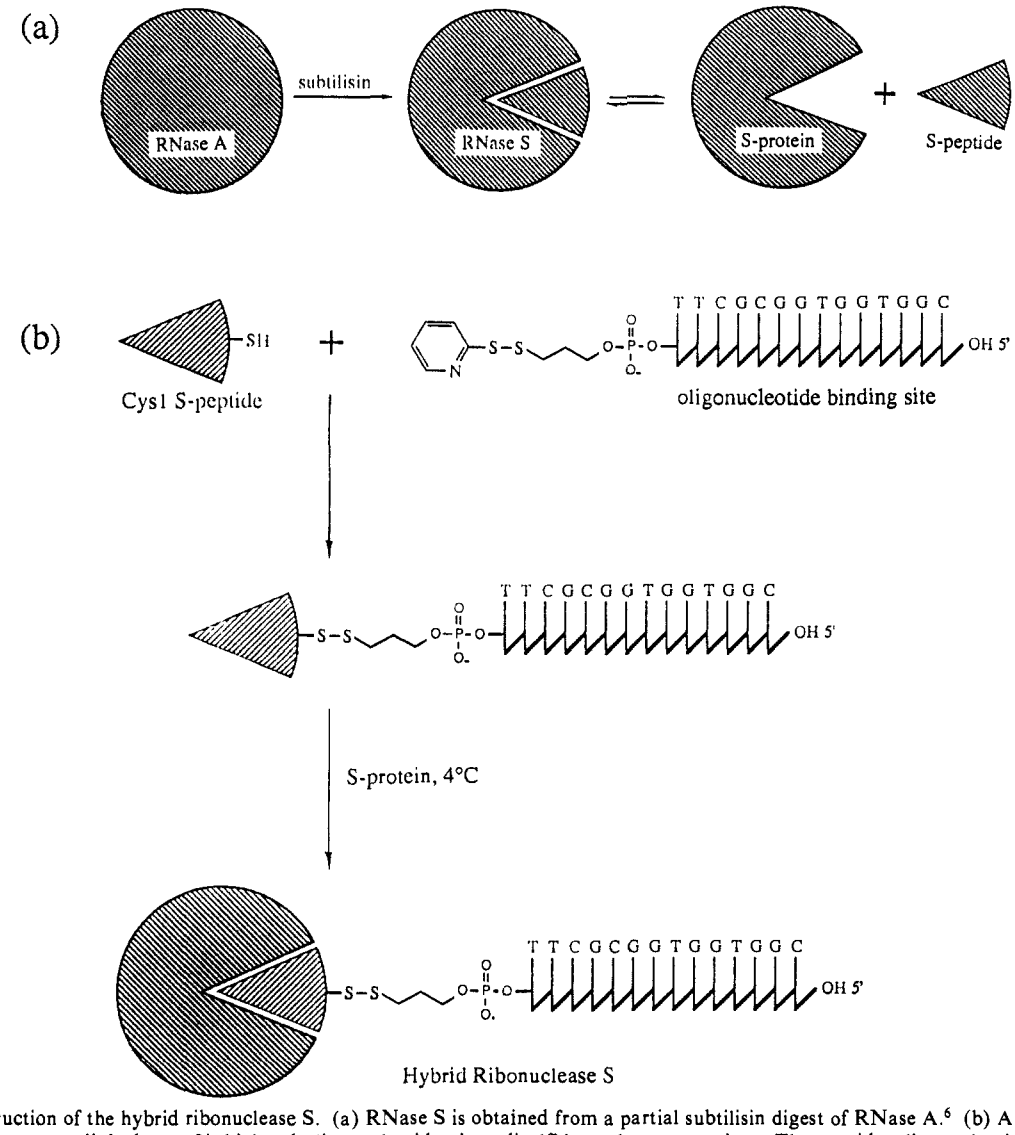

Figure 1. Construction of the hybrid ribonuclease S. (a) RNase S is obtained from a partial subtilisin digest of RNase A.[6] (b) A synthetic S-peptide (Lys1 to Cys1) was crosslinked to a 3′-thiolated oligonucleotide via a disulfide exchange reaction. The peptide–oligonucleotide adduct was then reconstituted with natural S-protein to afford the hybrid ribonuclease S.

of pyrimidines.[3] The X-ray crystal structure of ribonuclease A suggests that coupling of the 3′ terminus of an oligodeoxyribonucleotide to lysine 1 of RNase A should deliver a hybridized RNA to the enzyme active site.[4] The oligonucleotide binding site 5′-CGGTGGTGGCGCTT-3′ (which is resistant to RNase hydrolysis) was coupled to RNase A via a disulfide exchange reaction, a strategy which requires introduction of a free thiol at the amino terminus of RNase A and the 3′-terminus of the oligonucleotide.[1,2] A flexible tether was incorporated to allow some variability in the alignment of hybridized substrate with active site residues. We have previously adapted existing methods for solid-phase phosphoramidite synthesis of DNA oligomers to allow rapid incorporation of a 3′-*S*-thiopyridyl disulfide.[5] A thiol was introduced at the amino terminus of the enzyme by exploiting the fact that RNase A can be proteolytically cleaved into two fragments, the S-peptide (residues 1–20) and S-protein (residues 21–124), which can be recombined to form a tightly associated complex with full enzymatic activity (RNase S).[6] An S-peptide analogue containing a Lys1 to Cys1 substitution was synthesized by solid-phase Merrifield synthesis and coupled via a disulfide exchange reaction to the 3′-*S*-thiopyridyl oligonucleotide.[7] The adduct was isolated by anion exchange chromatography and recombined with natural S-protein to generate the hybrid enzyme which was purified by anion exchange chromatography[8] (Figure 1).

The ability of the hybrid enzyme to site-selectively cleave RNA was assayed with a 62-nucleotide single-stranded RNA prepared

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(7) Ten milligrams of the crude deprotected 20mer (obtained from Dr. Olshefski, UC San Diego) was treated with DTT (65 mM) in 1.0 mL of 500 mM Tris·Cl, 2 mM EDTA, pH 8.0 at 37 °C for 1 h. The thiol-containing peptide was purified by reverse-phase chromatography on a Pharmacia PepRPC HR 5/5 column with a gradient of 12–65% B in 25 min (flow rate = 1.0 mL/min, solvent A = 50 mM phosphate buffer, pH 2.7, solvent B = acetonitrile). The peptide (130 mmol) was combined with the 3′-*S*-thiopyridyl oligonucleotide (130 mmol) in 2.0 mL of 100 mM triethylammonium acetate buffer, pH 7.5. Formation of the crosslinked adduct was observed by monitoring the release of the thiopyridyl anion at 343 nm and was 95% complete in 20 min.
(8) The peptide–oligonucleotide adduct was purified by anion-exchange chromatography on a Pharmacia Mono Q HR 5/5 column with a gradient of 20–65% B in 18 min (flow rate = 1.0 mL/min, A = 20 mM NaOAc, pH 6.0/20% acetonitrile, B = A + 1 M KCl). The adduct was then desalted on Sephadex G-25. A portion of the desalted adduct (0.44 nmol) was incubated with a 10-fold molar excess of S-protein in 115 μL of 100 mM Tris·Cl, pH 7.5 at 37 °C for 1 h followed by 1 h at 4 °C to reconstitute the hybrid RNase S. Anion-exchange chromatography at 4 °C (Mono Q, 10–65% B in 20 min; flow rate = 1.0 mL/min; A = 20 mM Tris·Cl, 2 mM EDTA, pH 7.5; B = A + 1 M KCl) indicated 90% reconstitution based on disappearance of the peptide–oligonucleotide adduct.

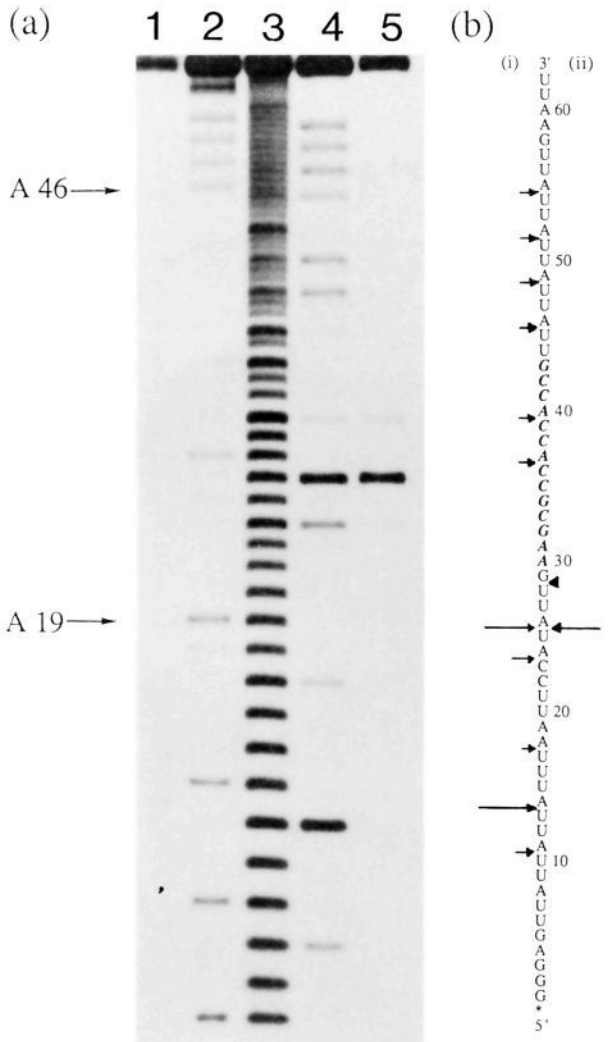

Figure 2. (a) Autoradiogram of a 25% denaturing polyacrylamide gel showing site-selective cleavage of a 5′ end-labeled 62mer of RNA. Lane 1, RNA only; lane 2, partial RNase U2 digest (A specific); lane 3, hydroxide cleavage ladder; lane 4, RNase S digest (in 20% formamide buffer); lane 5, hybrid enzyme digest: to 7 μL of a solution of end-labeled RNA (1.6 pmol) containing 2 μL deionized formamide in 50 mM Tris·Cl, 50 mM NaCl, 0.1 mM EDTA, pH 7.0, was added 3 μL of the hybrid enzyme solution (1.0 pmol) at 0 °C. The reaction was quenched after 3 s by the addition of 12 μL of formamide preheated to 95 °C. (b) Histogram indicating the cleavage pattern of (i) RNase S and (ii) the hybrid enzyme. The heights of the arrows indicate the relative cleavage intensities at the indicated bases, and the italicized sequence designates the oligonucleotide binding site.

by runoff transcription.[2] The transcript was end-labeled on the 5′ terminus with γ-^{32}P ATP and T4 polynucleotide kinase and purified on a 15% denaturing polyacrylamide gel. Cleavage of the end-labeled RNA was analyzed by high resolution denaturing polyacrylamide gel electrophoresis (Figure 2a).

Inspection of the histogram of the cleavage patterns produced by the hybrid enzyme (Figure 2b) reveals that the oligonucleotide binding domain site-selectively delivers the catalytic activity of RNase S to RNA. Cleavage occurs primarily at one pyrimidine–purine site adjacent to the site of oligonucleotide hybridization. In contrast, cleavage of RNA by RNase S, in the presence or absence of the free 14 nucleotide oligomer, occurs relatively nonselectively at 5′-pyrimidine–purine-3′ sites, UpA being the preferred cleavage site. These results demonstrate that the rate of hybridization and subsequent cleavage by the hybrid ribonuclease is more rapid than the rate of nonselective RNase S cleavage. High cleavage selectivity has thus been obtained by combining the specificity of the oligonucleotide with the inherent specificity of RNase S. Addition of S-protein to a preformed *S*-peptide–oligonucleotide RNA complex did not afford selective cleavage. Unfortunately, hybridization and cleavage by the hybrid enzyme at elevated temperatures (>37 °C) leads to diminished cleavage efficiency and specificity, most likely due to dissociation of the S-protein from the peptide–oligonucleotide adduct. Direct fusion of an oligonucleotide binding site to RNase A either via a bifunctional crosslinker or by construction of a Cys1 RNase A mutant may lead to hybrid enzymes capable of catalytically cleaving RNA's at any predefined site.

In conclusion, this work not only helps define chemical strategies for rationally altering enzymic properties but also may lead to a new class of sequence-specific ribonucleases for studying RNA structure and function.

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New Channel-Type Inclusion Compound of Steroidal Bile Acid. Structure of a 1:1 Complex between Cholic Acid and Acetophenone

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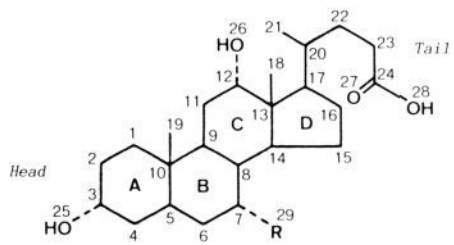
Special attention has been drawn recently to crystal inclusion chemistry which involves a variety of viewpoints.[2,3] Among lattice-type inclusion compounds,[3] typical steroidal bile acids, deoxycholic acid (DCA), and apocholic acid (ACA) are classical compounds in host–guest chemistry.[4] The crystal structures of DCA and ACA with guest molecules provide tunnel-like spaces usually called "channels".[5,6] Many crystallographic studies have indicated that the DCA channels can accommodate a wide variety of organic molecules[5] and also an organometallic compound such as ferrocene.[7] It is interesting to use the host–guest interaction in this channel as a model for reaction space in the solid state such as novel host–guest reactions[8] and inclusion polymerizations.[9]



Deoxycholic acid (DCA) R=H
Apocholic acid (ACA) R=H (8,14 en)
Cholic acid (CA) R=OH

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