When the different crystal forms of prednisolone *tert*-butylacetate are exposed to air and UV light for 30 days, only the hexagonal form (form V) degrades. This result is consistent with our previous studies.[4] As with cortisol *tert*-butylacetate, we hypothesize that the hexagonal form is most reactive because of the large tunnel running down the hexagonal axis as shown in Figure 4. The other forms are not reactive presumably because the oxygen penetrability is lower. The monoclinic ethanol solvate is an interesting case because is appears to contain solvent tunnels but is not reactive (see Figure 3). Careful analysis (using X-ray powder diffraction) of the desolvation of this solvate (which is presumably a prerequisite to oxygen penetration) shows that upon desolvation it transforms to the close-packed orthorhombic form. These results show that the crystal structure controls the reactivity of the prednisolone *tert*-butylacetate, a result similar to that obtained for the oxidation of hydrocortisone *tert*-butylacetate.[4]

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Registry No. Preanisolone *tert*-butylacetate, 7681-14-3.

Supplementary Material Available: Tables of intermolecular distances (Table III), bowing angles (Table IV), ^{13}C chemical shifts (Table V), and atomic parameters for prednisolone 21-*tert*-butylacetate and Figure 6 (interrupted decoupled spectrum) (10 pages). Ordering information is given on any current masthead page.

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Communications to the Editor

Site-Selective Cleavage of RNA by a Hybrid Enzyme

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The design of molecules capable of sequence specifically hydrolyzing large RNA's would greatly facilitate studies of RNA structure and function. Current strategies for selectively cleaving RNA include the use of chimeric oligonucleotides to direct RNase H cleavage[1] and cleavage by catalytic RNA's.[2] We report here the cleavage of RNA by a hybrid enzyme,[3] constructed by selectively introducing an oligonucleotide binding site into the relatively nonspecific phosphodiesterase, staphylococcal nuclease.[4] The Watson–Crick base pairing interactions of the oligonucleotide binding domain selectively deliver the hydrolytic activity of the nuclease to defined target sites on RNA as well as single-stranded DNA.[3]

The hybrid enzyme was constructed via a disulfide exchange reaction[5] between Cys116 of a mutant staphylococcal nuclease (K116 to C116)[3] and a 14-nucleotide oligomer containing a 3′-*S*-thiopyridyl disulfide.[6] A flexible tether was incorporated to allow some variability in the alignment of hybridized substrate with the active site residues.[4] The oligonucleotide–nuclease adduct was isolated by anion exchange chromatography in 90% yield[7]

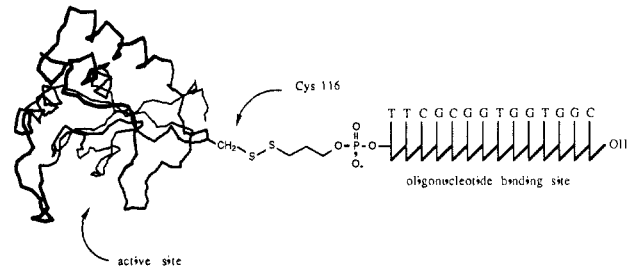

Figure 1. Schematic showing the alignment of the oligonucleotide binding site with the active site of staphylococcal nuclease (α-carbon backbone shown).

and was stable in the absence of Ca^{2+} ions.[8]

The ability of the hybrid enzyme to site specifically cleave RNA was assayed with a 59-nucleotide single-stranded RNA (Figure 2). The oligonucleotide binding site (T_m = 60 °C)[9] should direct the phosphodiesterase activity of the hybrid enzyme to the 5′ side of the complementary RNA sequence. The RNA substrate was synthesized by runoff transcription with T7 RNA polymerase[10,11] from EcoRI linearized plasmid pRNZ9. Plasmid pRNZ9 was constructed by ligating a synthetic 76 base pair fragment of duplex DNA (containing the 59-nucleotide sequence directly to the 3′-side of the T7 consensus promoter 5′-TAATACGACTCACTATA-3′) into HindIII/EcoRI digested pUC9.[12-14] The transcript was then labeled separately on either the 5′ or 3′ termini by using γ-^{32}P ATP and T4 polynucleotide kinase[12] or ^{32}P pCp and RNA ligase[15] and further purified on a 20% denaturing polyacrylamide gel.[16]

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(4) (a) Tucker, P.; Cotton, F.; Hazen, E. *Mol. Cell. Biochem.* **1978**, *22*, 67. (b) Tucker, P.; Cotton, F.; Hazen, E. *Ibid.* **1979**, *23*, 3. (c) Tucker, P.; Cotton, F.; Hazen, E. *Ibid.* **1979**, *23*, 67.
(5) Dimeric enzyme (160 nmol) in 5.0 mL of 2 mM Hepes, 50 mM NaCl, pH 6.8, was reduced to the monomer by treatment with 50 mM dithiothreitol for 12 h at 37 °C. The monomeric enzyme was purified by cation exchange chromatography on a Mono S HR5/5 column (Pharmacia) eluting with a linear gradient: 5% B for 20 mL, 5–65% B in 25 mL; A = 2 mM EGTA, 50 mM Hepes, pH 7.6; B = A + 1 M KCl; flow rate = 1.5 mL/min. The purified enzyme (150 nmol) was reacted with the 3′-*S*-thiopyridyl disulfide oligonucleotide (75 nmol) in 3.9 mL of column buffer containing 10 mM pTp. Formation of the crosslinked adduct was observed by monitoring the release of thiopyridyl anion at 343 nm and was 95% complete in 30 min.
(6) Zuckermann, R.; Corey, D.; Schultz, P. *Nucleic Acids Res.* **1987**, *15*, 5305.
(7) The adduct was purified by anion exchange chromatography on the Pharmacia Mono Q HR5/5 column with a gradient of 20–60% B in 18 min: A = 20 mM Tris·HCl, 2 mM EGTA pH 7.5; B = A + 1 M KCl; flow rate = 1.0 mL/min. The adduct was then desalted on Sephadex G-25 and stored in 5 mM Tris·HCl, 1 mM EGTA, pH 7.5.
(8) Cautrecasas, P.; Fuchs, S.; Anfinsen, C. *J. Biol. Chem.* **1967**, *242*, 1541.
(9) Calculated at 50 mM NaCl: Freier, S.; Kierzek, R.; Jaeger, J.; Sugimoto, N.; Caruthers, M.; Neilson, T.; Turner, D. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 9373.
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(11) The crude transcription mixture was extracted with 1:1 phenol/chloroform, followed by chloroform, after which the aqueous layer was loaded directly on the Mono Q HR5/5 anion exchange column (Pharmacia). The product RNA was readily separated from the NTP's by eluting with the gradient: 25–100% B in 18 min; A = 20 mM sodium phosphate/20% acetonitrile pH 6.0, B = A + 1 M KCl; flow rate = 1.0 mL/min.
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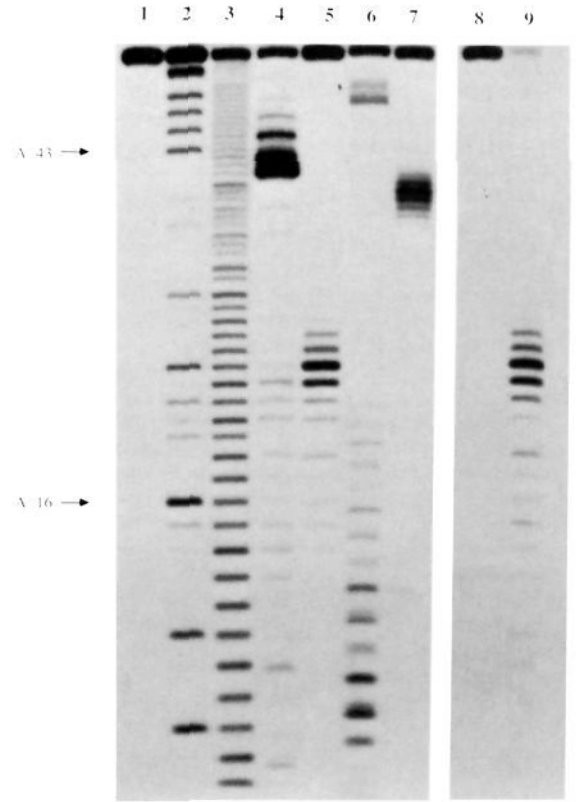

Figure 2. Autoradiogram of 25% denaturing polyacrylamide gel: lane 1, 5′ end-labeled (*) RNA only; lane 2, 5′* RNase U2 digest (A specific); lane 3, 5′* hydroxide cleavage ladder; lane 4, 5′* Cys 116 mutant digest; lane 5, 5′* hybrid enzyme digest; lane 6, 3′* Cys 116 mutant digest; lane 7, 3′* hybrid enzyme digest; lane 8, 5′* RNA only; lane 9, 5′* hybrid enzyme digest (excess enzyme). Hybrid enzyme cleavage conditions: To a 100 nM solution of either 5′ or 3′ end-labeled RNA in 50 mM Tris·HCl, 50 mM NaCl, 0.1 mM EDTA, pH 7.0, was added the hybrid nuclease (10 nM to 1 μM) to give a final volume of 9 μL. Hybridization was carried out by heating the mixture to 65 °C for 90 s and then cooling at 15 °C for 2 min. The cleavage reaction was initiated by the addition of 1 μL of 100 mM $CaCl_2$ at 15 °C and quenched after 1 s by the addition of 10 μL of formamide containing 10 mM pTp.

Figure 3. Histogram indicating the cleavage pattern of (a) the hybrid enzyme and (b) the underivatized Cys 116 mutant (from lane 4, Figure 2). The heights of the arrows indicate relative cleavage intensities at the indicated bases.

Cleavage reactions were performed by first incubating the hybrid enzyme with the RNA to form the DNA·RNA hybrid and then activating the nuclease by the addition of Ca^{2+} (the enzyme is completely dependent on Ca^{2+} for activity).[8] Cleavage of the end-labeled RNA by the hybrid enzyme was analyzed by high resolution denaturing polyacrylamide gel electrophoresis[16] (Figure 2).

Inspection of the histogram of the cleavage patterns produced by the hybrid enzyme (Figure 3) reveals that the oligonucleotide binding domain site selectively delivers the hydrolytic activity of staphylococcal nuclease to the RNA. Cleavage occurs over a 3 to 5 nucleotide region directly adjacent to the hybridization site. In contrast, cleavage of the RNA by staphylococcal nuclease (K116 to C116), in the presence or absence of the 14-nucleotide oligomer, occurs relatively nonspecifically at A-U rich regions of the RNA and at a much slower rate. The fact that the hybrid enzyme cleaves a number of phosphodiester bonds adjacent to the binding site may result from the flexibility of the tether linking the oligonucleotide to the enzyme. Cleavage of only one phosphodiester bond may result with a shorter or more rigid tether. Greater than 90% selective conversion of the RNA has been achieved with an excess of enzyme relative to substrate (lanes 8 and 9, Figure 2).[17] Cleavage of RNA without hybridization of the enzyme prior to the addition of Ca^{2+} also results in selective cleavage, suggesting that nonselective cleavage by the hybrid enzyme is relatively slow. At longer reaction times or elevated temperatures, the cleavage specificity decreases, possibly due to autolysis of the hybrid enzyme.

In conclusion, this work illustrates an approach for rationally modifying enzyme specificity which may not only lead to a class of selective ribonucleases but may also find application in designing additional selective catalysts.

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(17) Determined by densitometry of the autoradiogram in Figure 2.

Difference in the Methyl Group Conformational Preferences of T_1 and S_1 Acetophenone. A Probe of the Orbital Character of Excited Electronic States

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Ethane exists, in its electronic ground state, primarily in the staggered form because of the mutual repulsion between the filled π_{CH_3} orbitals of the two methyl groups in the higher energy eclipsed form. The same considerations dictate the more stable conformation of a methyl rotor attached to an R(-C)=X group, as in propene (R = H, X = CH_2), acetaldehyde (R = H, X = O), and acetophenone (R = ϕ, X = O), all of which have a C–H bond eclipsed (**1**) rather than staggered (**2**) with the double bond in

the ground state (S_0). But, because of the sensitivity of these nonbonding interactions to the orbital occupancies of neighboring groups, the situation might be expected to be different in electronically excited states.[1] We report here on a realization of this expectation, show that a *difference* exists in the conformational preferences of the methyl group in the lowest excited triplet (T_1) and singlet (S_1) states of acetophenone, *probably because of a difference in their orbital characters*, and comment briefly on the dynamic implications of this result.[2]

Our experiments were performed in the collision-free environment of a seeded supersonic jet of helium, by using a Nd^{3+}:YAG pumped and doubled dye laser as the excitation source. Acetophenone was directly excited to the T_1 (or S_1) state, and the $T_1 \leftarrow S_0$ (or $S_1 \leftarrow S_0$) excitation spectrum was recorded by monitoring the total phosphorescence (or fluorescence) intensity

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[‡] Montana State University.
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