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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 it 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.

(37) Van der Hart, D. L. *J. Magn. Reson.* **1981**, *44*, 117.

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

Acknowledgment. This research was supported by NIH Grant GM34520. The solid state NMR spectra were first measured at Colorado State University on a Nicolet 150 spectrometer (supported by NSF Grant CHE 78-18581).

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

Supplementary Material Available: Tables of intermolecular distances (Table III), bowing angles (Table IV), ¹³C 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.

Communications to the Editor

Site-Selective Cleavage of RNA by a Hybrid Enzyme

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Received September 23, 1987

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 cleavage¹ 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 reaction⁵ 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% yield⁷

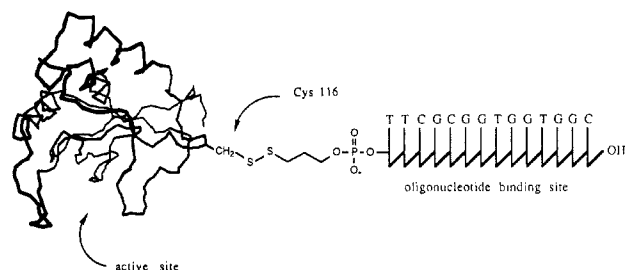


Figure 1. Schematic showing the alignment of the oligonucleotide binding site with the active site of staphylococcal nuclease (α -carbon backbone shown).

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 ($T_m = 60^\circ\text{C}$)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 -³²P ATP and T4 polynucleotide kinase¹² or ³²P pCp and RNA ligase¹⁵ and further purified on a 20% denaturing polyacrylamide gel.¹⁶

(1) Shibahara, S.; Mukai, S.; Nishihara, T.; Inoue, H.; Ohtsuka, E.; Morisawa, H. *Nucleic Acids Res.* **1987**, *15*, 4403.

(2) Cech, T. *Science (Washington, D.C.)* **1987**, *236*, 1532.

(3) Corey, D.; Schultz, P. *Science (Washington, D.C.)*, in press.

(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 $^\circ\text{C}$. 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.

(10) (a) "Transcription Systems" (Chapter 2) in the Promega Biotec 1986/87 catalog, p 11. (b) Uhlenbeck, O. *Nature (London)* **1987**, *328*, 596.

(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.

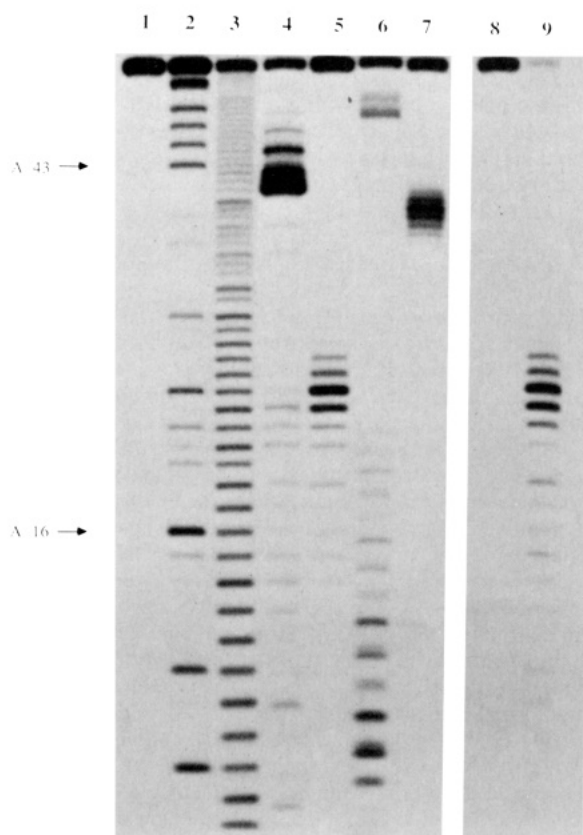
(12) Maniatis, T.; Fritsch, E.; Sambrook, J. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory: New York, 1982.

(13) Holmes, D.; Quigley, M. *Anal. Biochem.* **1981**, *114*, 193.

(14) Colpan, M.; Reisner, D. J. *Chromatogr.* **1984**, *296*, 339.

(15) Uhlenbeck, O.; England, T. *Nature (London)* **1978**, *275*, 560.

(16) Maxam, A.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 560.



(a) 5'-GGGAGUUAUUAUUUUUUUUAUUAUUAUUAAGCGCGCACCGUUAUUUUUUUUUUUUAUUU-3'

(b) ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑

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 electrophoresis¹⁶ (Figure 2).

phodiester 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.

Acknowledgment. Supported in part by the Camille and Henry Dreyfus Foundation and by a Presidential Young Investigator Award (NSF CHE85-43106). We are grateful to C. Noren for providing T7 RNA polymerase and to Dr. J. Buzayan for providing assistance with runoff transcription.

Difference in the Methyl Group Conformational Preferences of T₁ and S₁ 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 $\text{R}(\text{-C})=\text{X}$ group, as in propene ($\text{R} = \text{H}$, $\text{X} = \text{CH}_2$), acetaldehyde ($\text{R} = \text{H}$, $\text{X} = \text{O}$), and acetophenone ($\text{R} = \phi$, $\text{X} = \text{O}$), all of which have a C–H bond eclipsed (**1**) rather than staggered (**2**) with the double bond in



Our experiments were performed in the collision-free environment of a seeded supersonic jet of helium, by using a Nd³⁺:YAG pumped and doubled dye laser as the excitation source. Acetophenone was directly excited to the T₁ (or S₁) state, and the T₁ ← S₀ (or S₁ ← S₀) excitation spectrum was recorded by monitoring the total phosphorescence (or fluorescence) intensity

(2) A preliminary report of this work was given at the 41st Symposium on Molecular Spectroscopy, Columbus, OH, June, 1986 (abstract RD'5).