In **1**, **2**, and **5**, hyperconjugative interactions destabilize the π_+ orbital relative to the π_- orbital, causing the frontier orbitals to become nearly degenerate and leading to T ground states. In **6**, the π_+ and π_- orbitals are destabilized to nearly the same extent by hyperconjugative interactions, and it is the twisting of the TME group that decreases the π_+,π_- splitting and leads to a T ground state. In **3** and **4**, the resonance interactions with the heteroatoms are sufficiently large that the π_+ orbital is "pushed" above π_-, leading to orbital splittings greater than that in planar TME and resulting in S ground states.

In a two-orbital model, the S–T splitting can be expressed as

$$\Delta E = E_T - E_S = J_{12} - 0.5(J_{11} + J_{22}) + 0.5(\epsilon_1 - \epsilon_2)^2/K_{12} \quad (1)$$

where ϵ_1 and ϵ_2 are the frontier orbital energies and J and K represent the Coulomb and exchange interactions, respectively, and where it has been assumed that $K_{12} \gg |\epsilon_1 - \epsilon_2|$. This expression is derived from eqs 16 and 18 of ref 1b.

Figure 1 plots the MCSCF(2,2) and CI+DV2 S–T gaps vs $(\epsilon_{\pi+} - \epsilon_{\pi-})^2$ as well as straight-line fits of the data for the five-membered-ring systems, **1**–**5**. Although eq 1 is derived for a two-electron model, the scatter of the data points from the straight-line fits is small. The 0.4–1.0 kcal/mol deviations of the points for TME and **6** from the straight lines is due, in part, to the nonplanarity of **6** and to the variations of the exchange integrals across the series.

This study shows that, for a series of diradicals such as **1**–**5**, the S–T splittings display an approximate quadratic dependence on $(\epsilon_{\pi+} - \epsilon_{\pi-})$, even when calculated employing methods that recover electron correlation. Since the trends in the orbital splittings can be interpreted in terms of orbital interactions, simple MO calculations can be used to design diradical systems with desired S–T gaps.

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Hammerhead Ribozyme Tertiary Folding: Intrinsic Photolabeling Studies

Anne Woisard and Alain Favre*

Laboratoire de Photobiologie Moléculaire
Institut Jacques Monod, CNRS
2, Place Jussieu, 75251 Paris Cedex 05, France

Pascale Clivio and Jean-Louis Fourrey*

Institut de Chimie des Substances Naturelles
CNRS, 91198 Gif sur Yvette Cedex, France

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The class of hammerhead ribozymes includes artificial RNA constructs which, when properly designed, exercise endonuclease activity on their RNA substrate.[1] In this case, the ribozyme–substrate complex (R–S) forms a hammerhead-shaped secondary structure containing the conserved nucleotides which have been identified in various self-cleaving RNAs, in viroids, and in satellite RNAs of plant viruses.[2] It is likely that the cleavage reaction requires a well-defined tertiary structure for R–S whose determination will demand much research effort.[3] In this respect, we have formed a hammerhead domain between a 35-mer ribozyme (R) and a 14-mer substrate (S) exhibiting high catalytic activity (Scheme I). Reported herein is our exploration of the tertiary folding of this system by application of a labeling technique which makes use of the photo-cross-linking properties of the newly designed intrinsic probe, 2′-deoxy-6-thioinosine (ds^6I).

Scheme I. Design of the 35-mer Ribozyme R and Its 14-mer Substrate S according to Ref 1b[a]

CLEAVAGE

5′ U G C C C **G U C** ↓ U G U U G U 3′ Substrate **S**

T G C C C **G** s^6**I** **C** T G T T G T — Substrate Analogue **X**

3′ A C G G G **C A** ... A C A A C A 5′ — Ribozyme **R**

A A G C—G A G A U C U G A-10 U; A—U; G—C; G—C; A G; G U (20)

[a] R contains a 24-nucleotide sequence in common with the (+) strand of the satellite RNA of tobacco ringspot virus (sTobRV RNA), i.e., the consensus sequences and stem III. S corresponds to a conserved sequence, between residues 107 and 121, of the U5 region in the long terminal repeat (LTR) of HIV RNA. The photoactivable DNA substrate analogue X is sequentially identical to S except for 2′-deoxy-6-thioinosine at position 7 and thymidine in place of uridine. These oligonucleotides were chemically synthesized. Standard Watson–Crick base pairs are represented by —. The O symbol between ds^6I and A represents for the nonstandard base pair possibly formed between these residues. The kinetic data for the cleavage reaction of S were found to be k_{cat} = 25 min^{-1} at pH 8 (50 mM Tris-HCl), 37 °C, 25 mM Mg^{2+}.

The preliminary photochemical studies, performed with thymidylyl-(5′–3′)-2′-deoxy-6-thioinosine (**1**) ($Tpds^6I$),[4] showed the remarkable capacity of ds^6I to undergo covalent bonding with a pyrimidine residue. When **1** was exposed to UV light (366 nm), the major photoproduct **2** (λ_{max} 271 nm) was formed in good yield (Scheme II). Structure **2** was established after examination of the spectral data. The molecular formula ($C_{20}H_{25}N_6O_{10}PS$) resulted from HR FAB MS. The ^1H NMR spectrum of the (6–6) adduct **2** displays at 5.64 ppm a characteristic signal due to the H_6 of its saturated Tp unit. Such a chemical shift is consistent with that of the corresponding proton in related (6–4) pyrimidine pyrimidinone photoproducts.[5] Other key NMR arguments were

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(4) See supplementary material for details on the synthesis (Clivio, P.; Fourrey, J.-L.; Gasche, J.; Favre, A. *Tetrahedron Lett.* **1992**, *33*, 65–68) and irradiation of **1** and on the characterization of compounds **1** and **2**.

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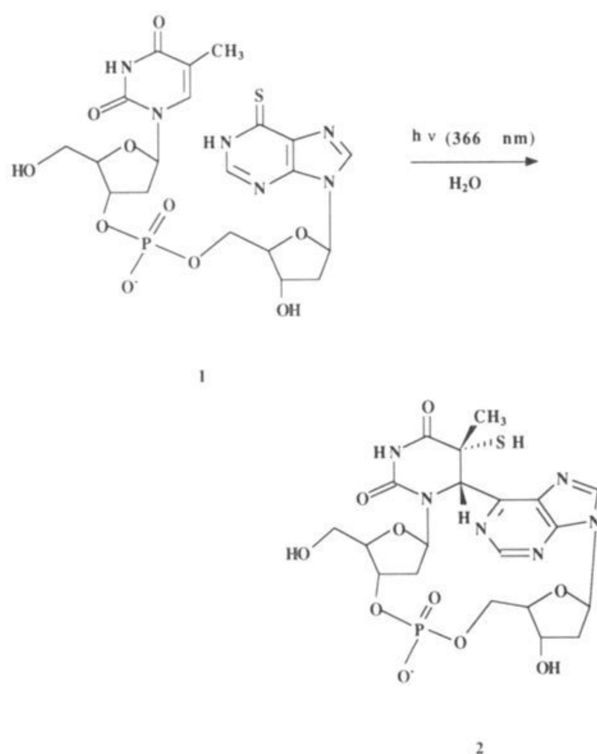
Scheme II. Photochemical Reaction of Tpds^{6}I (**1**) To Give **2**



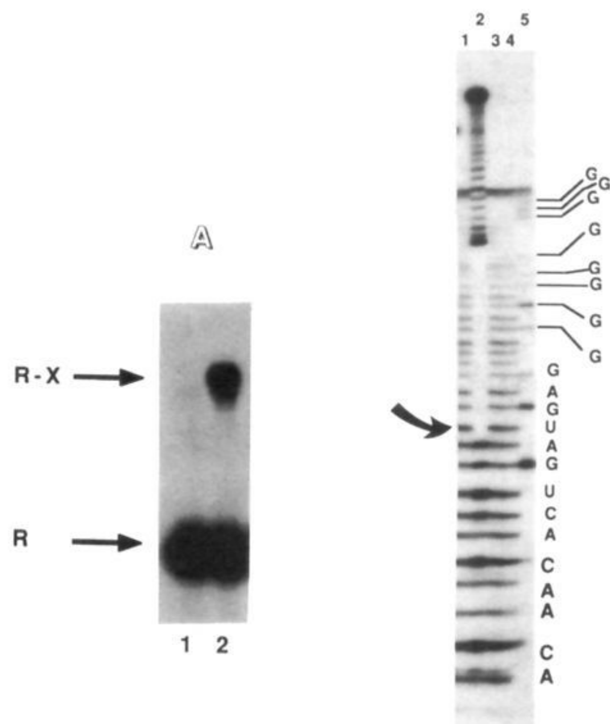


Figure 1. Isolation and identification of the cross-linked species R–X. (A) Autoradiogram of the separation polyacrylamide (15%) gel in 7 M urea. Lane 1: 5′-^{32}P-labeled R irradiated in the absence of X. Lane 2: 5′-^{32}P-labeled R (0.4 μM) submitted to the same treatment in the presence of unlabeled X (1.4 μM). (B) Autoradiogram of the sequencing gel. Lanes 1, 3, and 4 represent the limited basic hydrolysis of control 5′-^{32}P-labeled R. Lane 2 shows the limited basic hydrolysis of the R–X complex. Lane 5 corresponds to limited RNase T digestion of control R. The arrow indicates the cross-linked position.

obtained from the ^{13}C NMR data, which showed that the C_5 and C_6 carbons of the purine part are sp^2 hybridized since they resonate at 157.6 and 134.7 ppm, respectively.[6] Finally, C_5 and C_6 were attributed an *R* and an *S* configuration, respectively, and the two bases were shown to be in anti conformation, on the basis of NOE measurements.[7]

These observations prompted us to use ds^{6}I as a probe for photolabeling experiments directed at deciphering tertiary folding in the hammerhead ribozyme series. Since there is strong evidence that an uncleavable full DNA substrate can be used in hammerhead ribozyme structural studies,[8] we have prepared the substrate analogue (X) (Scheme I) incorporating ds^{6}I at position 7 next to the cleavage site. Then X (1.4 μM) and 5′-^{32}P-labeled R (0.4 μM) were combined in the buffer used to observe the cleavage reaction within R–S. The resulting solution was irradiated at 366 nm, and direct evidence for an efficient photo-cross-linking reaction (25%)[9] between R and X was provided by gel electrophoresis, which revealed one strongly retarded spot (Figure 1A). The cross-linked species (R–X) was eluted, and a method to identify the residue of the ribozyme which had bound to ds^{6}I was developed in the following manner. When 5′-^{32}P-labeled R was submitted to a limited alkaline digestion and the resulting mixture electrophoresed, a ladder was observed as expected. However, when this procedure was applied to R–X, the same partial ladder was generated by cleavage of the phosphodiester bonds located on the 5′ side of the cross-linked unit whereas all the cleavage products arising from the 3′ side of this residue remained attached to the alkaline resistant 14-mer deoxynucleotide. This resulted in a clear-cut window on the gel, allowing the unambiguous determination of the branching point, namely, uridine 11 of the ribozyme (Figure 1B).[10]

A salient question is whether the above cross-link found within R–X is representative of the actual folding of the R–S complex? According to Ruffner et al.,[11] standard base pairing between U7 of S and A29 of R (Scheme I) is crucial for activity; thus the presence of I at position 7 of the true substrate is expected to prevent cleavage. To answer this question, we have compared the ribozyme activity of R upon either the true substrate S or its analogues dS$_C$ and X$_C$ which contain a ribocytidine at the cleavage position[12] and are thus potentially cleavable.[13] Both dS$_C$ and X$_C$ were cleaved under the standard conditions with the initial rates being 50 and 25% of the one observed with the true substrate S, respectively. Hence the replacement of U by s^{6}I at position 7 results in a substrate with a lower but significant activity. It is very likely that the new probe ds^{6}I (related to 4-thiouridine for its photochemical behavior[14]) has revealed a characteristic folding of the hammerhead ribozyme domain with U$_{11}$ of R coming very close to the cleaving site. Hopefully, the herein reported observations might help future modeling studies of the hammerhead ribozymes.

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(6) C_6 was assigned from its long range correlations with both H_2 of the purine (^3J) and H_6 of the pyrimidine (^2J). Similarly C_5 was correlated with H_8 (^3J).

(7) The following sets of NOE were observed: TpH$_6$–Tp(CH$_3$); TpH$_6$–TpH$_{3'}$; TpH$_6$–TpH$_{5'}$H$_{5''}$; p(ds^{6}I)H$_8$–p(ds^{6}I)H$_{2'}$; p(ds^{6}I)H$_8$–p(ds^{6}I)H$_{3'}$.

(8) See ref 3c and references given therein. In this laboratory, further evidence was gained from competition experiments (data not given).

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Supplementary Material Available: Reaction scheme for the preparation of **1** and of the phosphoramidite derivative **6** used to incorporate ds[6]I in X, experimental conditions, and spectral data for compounds **1** and **2** (6 pages). Ordering information is given on any current masthead page.

Alkane Activation and Oxidative Addition to Rh by Photodesorption of Surface Carbonyl Ligands[1]

T. H. Ballinger and J. T. Yates, Jr.*

Surface Science Center, Department of Chemistry
University of Pittsburgh
Pittsburgh, Pennsylvania 15260

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The activation of the strong C–H bonds in alkanes (RH) by interaction with partially "naked" metal centers in complexes such as CpRh(CO)[2] readily occurs in the homogeneous phase, producing CpRh(CO)(H)R.[3-9] The active center is generated by photolysis of $CpRh(CO)_2$,[10-13] and the presence of the transient CpRh(CO) has just been demonstrated.[14] Such a reaction scheme can provide novel pathways for producing various organic compounds from inactive alkanes. A heterogeneous version of this process would be very desirable as a route to alkane activation.

This communication describes the use of a heterogeneous system, Rh/Al_2O_3, to achieve the same alkane activation chemistry. It is well-known that the chemisorption of CO on supported Rh produces isolated $Rh(CO)_2$ species.[15-23] This produces a characteristic carbonyl doublet in the infrared spectrum, as observed by transmission IR. Figure 1 shows IR spectral changes due to photodecomposition of the $Rh(CO)_2$ species by irradiation at 325 nm. The Rh surface is supported on an electrically heated grid in ultrahigh vacuum.[24] By variation of the electrical power input to the grid, care was taken to maintain a constant surface temperature (±1 K) during irradiation and in the dark. Figure 2 shows the kinetics of the UV photodecomposition process. Initially, in the dark, a slow rate of CO thermal desorption is observed. Upon irradiation, the rate of loss of $Rh(CO)_2$ is enhanced substantially. A control experiment, using identical power deposition in the catalyst from a tungsten–halogen lamp, follows

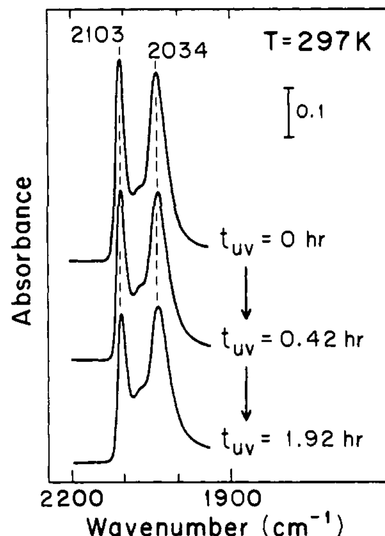

Figure 1. Photodecomposition of $Rh(CO)_2/Al_2O_3$ in vacuum.

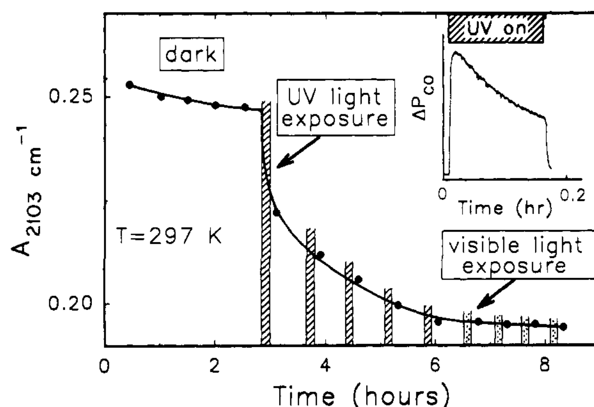

Figure 2. Kinetics of $Rh(CO)_2$ photodecomposition in vacuum by UV light (325 ± 50 nm) compared to visible light at equal power absorption as measured calorimetrically using the grid and thermocouple.

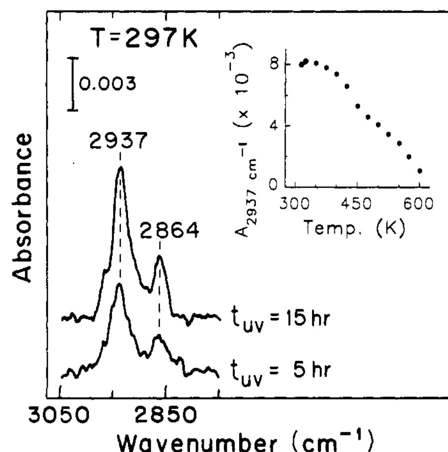

Figure 3. Photochemical production of chemisorbed cyclohexyl species from cyclohexane. Inset shows its thermal stability in vacuum.

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