for benzene chemisorbed on 95%-disperse Pt/η-Al_2O_3, a doublet indistinguishable from that observed in bulk benzene at temperatures >90 K (where rapid reorientation prevails) is observed at 80 K. At temperatures above 80 K (Figure 1a,b), an initially broad central component becomes apparent and grows in at the expense of the Pake doublet pattern. The spectrum becomes a single line at 160 K and rapidly narrows at higher temperature. This motional behavior has also been observed in a recent deuterium NMR study over a higher temperature range ($T > 75$ K) of a monolayer of benzene adsorbed on Pt/η-Al_2O_3 and has been attributed to fluctuations of the direction of the hexad rotational axis.[11,12]

In order to determine the carbon-carbon bond length, we measured the doublet splitting at 80 K over a wide range of rf duty factors for benzene-*1,2*-$^{13}C_2$ in the catalyst sample and found a C-C distance of 1.42 Å (uncorrected for vibrational motion[13]), the same result obtained for doubly-^{13}C-labeled benzene diluted in benzene with ^{13}C in natural abundance. This value is slightly larger than the 1.398 Å C-C distance in crystalline benzene determined by neutron diffraction,[14] but the difference may be accounted for in large part by a vibrational correction to the NMR splitting.

At temperatures below 80 K (Figure 1d-g), outer peaks appear with twice the splitting of the doublet observed at 80 K. This pair of doublets cannot be due to distorted benzene molecules with C-C bond lengths of 1.42 and 1.80 Å (i.e., $2^{1/3} \times 1.42$ Å). If this were the case, the intensities of the two doublets would be equal under all experimental conditions since polarization of the benzene protons, which determines the intensity of the signal, is independent of the distance between ^{13}C spins. The intensity behavior we observe is completely different, as shown in Figure 1e-g. When the recycle delay time is increased at constant temperature (Figure 1e,f), the relative intensity of the outer doublet increases, indicating a longer relaxation time for ^{13}C spins responsible for the larger splitting. This is consistent with assignment of the outer doublet to benzene rings moving slowly on the NMR time scale ($\sim 10^{-4}$ s). Moreover, as the temperature is lowered and the recycle delay time is kept constant (Figure 1e,g), the relatively intensity of the outer doublet again increases, indicating that the fractional number of static molecules is increasing. We conclude from both of these results that the inner and outer doublets arise from molecules on different sites which are rapidly reorientating or are static, a conclusion consistent with the 2:1 ratio of the splittings.

The observation of a single Pake doublet at 80 K does not rule out the possibility of a thermally-induced rotational average of two bond lengths with an average C-C distance of 1.42 Å. However, the single outer doublet observed at 6 K (Figure 1g), corresponding to static molecules, rules out that possibility and constitutes convincing evidence for the absence of such a distortion of the benzene ring in these samples.

We conclude that a distorted structure for benzene chemisorbed on a highly disperse Pt/η-Al_2O_3 catalyst, involving long and short C-C bonds, is not consistent with our experimental results. Although a rapid equilibration of structures of lower symmetry not involving a rotation of the whole molecule cannot be ruled out, some restrictions are suggested by our data. For example, the possibility of two C-C bonds with an average length considerably greater than that in bulk benzene as proposed for benzene co-adsorbed with CO on Pt(111)[15] would not be in agreement with our experimental results. The motional behavior of benzene chemisorbed on highly disperse Pt/η-Al_2O_3 is drastically different from that of bulk benzene, with rapid motion on the NMR time scale still present at temperatures even close to liquid helium.[16]

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(16) We have also performed measurements on a Rh/η-Al_2O_3 catalyst. The preliminary results reveal considerably different motional behavior from that of Pt.

Triple-Helix Formation by Oligodeoxynucleotides Containing the Carbocyclic Analogs of Thymidine and 5-Methyl-2′-deoxycytidine

Brian C. Froehler*

Gilead Sciences, Inc., 346 Lakeside Drive
Foster City, California 94404

Daniel J. Ricca

Glaxo Research Institute, 5 Moore Drive
Research Triangle Park, North Carolina 27709

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Sequence specific triple-helix formation of an oligodeoxynucleotide (ODN), via the parallel binding motif, requires recognition of 2′-deoxyadenosine by thymidine and recognition of 2′-deoxyguanosine (dG) by 2′-deoxycytidine.[1,2] Recognition of dG is complicated by the need to protonate the N-3 of the cytosine nucleobase to form a hydrogen bond with the N-7 of the guanine heterocycle.[3] 5-Methyl-2′-deoxycytidine has been shown to be a useful substitute for 2′-deoxycytidine,[4] and while it extends the pH range of triple-helix formation, protonation at physiological pH of this heterocycle is still problematic. A recent approach to overcome this problem has been the introduction of analogs which emulate the hydrogen-bonding face of a N-3-protonated cytosine without the need for protonation.[5] Another approach is to increase the pK_a of the 2′-deoxycytidine nucleobase and therefore facilitate protonation at neutral pH, but no analog of this type has been introduced. Formation of double-helix structures with the carbocyclic analog of polythymidylic acid have been reported,[6,7] but no triple-helix complexes were demonstrated. Reported herein

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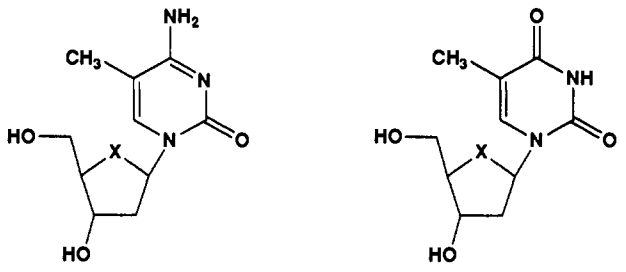

1; X = CH_2 3; X = CH_2
2; X = O 4; X = O

Duplex: 5' CTCAGAGAGAGAGAAAAAGGACCTC 3'
(25-mer) 3' GAGTCTCTCTCTCTTTTTCCTGGAG 5'

5; 5' TCTCTCTCTCTTTTT 3'
6; 5' TC*TC*TC*TC*TC*TTTTT 3'
7; 5' TCT*CT*CT*CT*CT*TTTT 3'

Figure 1. Structure of the carbocyclic analogs of 5-methyl-2′-deoxycytidine and thymidine. Duplex and third strand sequences for T_m analysis: C is 5-methyl-2′-deoxycytidine (**2**), C* is cmdC (**1**), T is thymidine (**4**), and T* is cT (**3**).

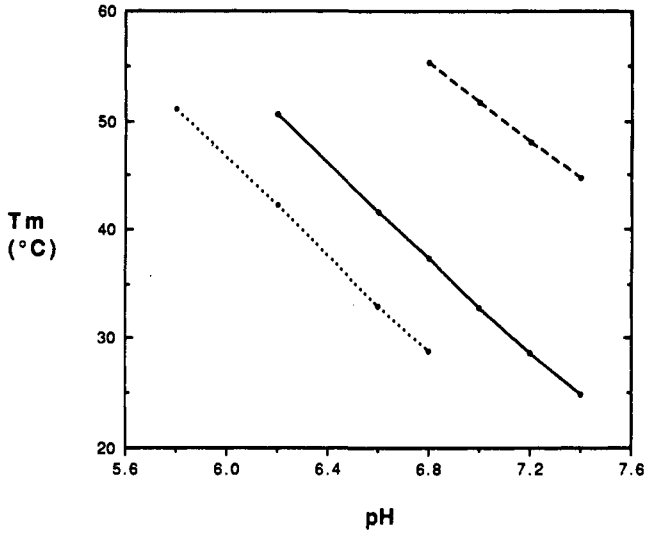

Figure 2. T_m vs pH plot for triple-helix-forming ODNs in 140 mM KCl/5 mM Na_2HPO_4/5 mM $MgCl_2$: (—) ODN **5**; (---) ODN **6**; (···) ODN **7**.

is the use of oligonucleotides derived from the carbocyclic analogs of the pyrimidines in triple-helix formation. The carbocyclic analog of 5-methyl-2′-deoxycytidine (cmdC, **1**) has a higher pK_a than the parent nucleoside (**2**), thus facilitating protonation at physiological pH and stabilizing the triple-helix complex.

The carbocyclic analog cmdC (**1**)[8] was prepared from carbocyclic thymidine (cT, **3**)[6,7c] by the $POCl_3$/triazole procedure.[9] These analogs differ from the natural nucleoside by substitution of the furanosyl oxygen with a methylene, generating a cyclopentane ring (Figure 1). Removal of the electron-withdrawing oxygen of the furanosyl ring has a significant effect on the basicity of the parent heterocycle. Titration of the hydrochloride salts of **1** and **2** shows that the pK_a of the carbocyclic analog **1** (4.80) is 0.45 units greater than the pK_a of the native furanosyl nucleoside **2** (4.35).[10,11]

The cytosine nucleosides (**1** and **2**) were N-4 functionalized with a benzoyl protecting group, and the 5′-dimethoxytrityl-protected nucleoside H-phosphonates of **1–4** were prepared by standard procedures[12] and used for oligonucleotide synthesis as previously described.[7,13] Separate ODNs were prepared from cmdC (**1**) and cT (**3**) containing five substitutions in a 15-mer (Figure 1).[14] The effect of the carbocyclic analogs on the stability of the triple-helix complex was assessed by thermal denaturation experiments with ODNs **5–7** as previously described.[15] ODN **6**, containing cmdC (**1**), results in an increase in Tm of 3.9 °C/substitution over ODN **5** (ΔT_m at pH 7.2 = +19.4 °C), and conversely, ODN **7**, containing cT (**3**), results in a decrease in T_m of 1.7 °C/substitution (ΔT_m at pH 6.6 = −8.7 °C) relative to ODN **5**. The pH dependence of triple-helix formation with these ODNs (**5–7**) was determined by a T_m versus pH plot.[15,16] With all ODNs the slope of the plot remained relatively constant (−18 to −20 °C/pH unit, Figure 2). The T_m of the underlying duplex was 68.6 ± 0.4 °C and showed no pH dependence. These results are consistent with a recent report describing triple-helix formation with 5-(1-propynyl)-2′-deoxycytidine.[17] This cytidine analog is less basic than 5-methyl-2′-deoxycytidine and shows a substantial decrease in T_m of the triple helix, but no effect on the pH dependence was observed.

An unexpected result is the low T_m of ODN **7** relative to ODNs **5** and **6**. This result would indicate that hydrophobic interaction of the carbocyclic nucleoside is probably not responsible for the increased T_m of ODN **6** and that the carbocyclic substitution alone may be deleterious to triple-helix formation. The crystal structure of **3** indicates a C1′-exo pucker of the cyclopentane ring,[7a] and this may be an unfavorable conformation for triple-helix formation.

These T_m results were confirmed by DNase footprint analysis[18] of a 370-bp restriction fragment containing the target sequence.[18c] The results demonstrate that cmdC (**1**) substitution, in place of mdC (**2**), leads to approximately a 100-fold increase in the stability of the complex at pH = 7.2.[19]

The carbocyclic analog of 5-methyl-2′-deoxycytidine (**1**) has been shown to stabilize the triple-helix complex relative to the furanosyl nucleoside; this effect is due in part to the increased basicity of the heterocycle (ΔpK_a = 0.45). Conversely, the carbocyclic analog of thymidine (**3**) decreases the stability of the triple-helix complex. Carbocyclic analogs of nucleosides exhibit greater enzymatic stability to cleavage of the glycosidic linkage[20] and therefore may be useful in therapeutic applications.

Acknowledgment. We thank Sarah McCurdy for ODN synthesis and purification, John Helms for base composition analysis,

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Supplementary Material Available: Autoradiogram derived from DNase footprint (1 page). Ordering information is given on any current masthead page.

The O_2-Evolving Center of Photosystem II Is Diamagnetic in the S_1 Resting State

Dionysios Koulougliotis, Donald J. Hirsh, and Gary W. Brudvig*

Department of Chemistry, Yale University New Haven, Connecticut 06511

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The O_2-evolving center (OEC) of photosystem II (PSII) catalyzes the four-electron oxidation of water to O_2. Although four Mn ions per PSII are required for O_2-evolution activity, the nuclearity and structure of the Mn cluster constituting the OEC are currently under debate.

We previously used EPR spectroscopy and O_2-consumption measurements to probe the nature of the change in the OEC occurring during dark adaptation.[1] The Mn site was found to exist in resting and active S_1 states depending on whether it was subjected to long (4 h) or short (6 min) periods of dark adaptation at 0 °C, respectively.

In this report, we address the effect of dark adaptation on the magnetic properties of the Mn site in the OEC by using the pulsed EPR method of saturation recovery.[2] We have shown in ribonucleotide reductase from *Escherichia coli*[3] that the spin-lattice relaxation of its stable tyrosine radical could serve as a probe of the magnetic properties of the EPR-silent dinuclear Fe(III) center. In this study, we probe the magnetic properties of the Mn cluster in PSII by analyzing the spin-lattice relaxation behavior of the stable tyrosine radical, $Y_D^{\bullet}$.

PSII membranes were prepared by the procedure of Berthold et al.[4] as modified by Beck et al.[1] The S_1 resting and active states were prepared according to Beck et al.[1]

The technique of saturation-recovery EPR has been used[5] to probe the magnetic interaction between the tyrosine radical ($Y_D^{\bullet}$) of PSII and the non-heme Fe(II) in Mn-depleted PSII. Saturation-recovery traces are analyzed according to a model[5] that takes into account the scalar exchange (isotropic) and the dipole-dipole (orientation dependent) interactions between two paramagnetic species separated by a fixed distance but with a random orientation with respect to the external magnetic field. The previously measured[5] dipolar rate constants, $k_{1dipolar}$, for $Y_D^{\bullet}$ in Mn-depleted PSII membranes are shown in Figure 1. It is observed that the rates are 4-5-fold faster in the S_1 active state over the whole temperature range (4-57 K). This is an initial indication that the S_1 active state of the Mn cluster is paramagnetic. However, in short dark-adapted PSII membranes, about 25% of the centers are still in the S_0 state, which has also been shown to be a good relaxer of the $Y_D^{\bullet}$ radical.[6] The question of the contribution of the S_0 state to the relaxation enhancement of $Y_D^{\bullet}$ in short dark-adapted PSII will be addressed below.

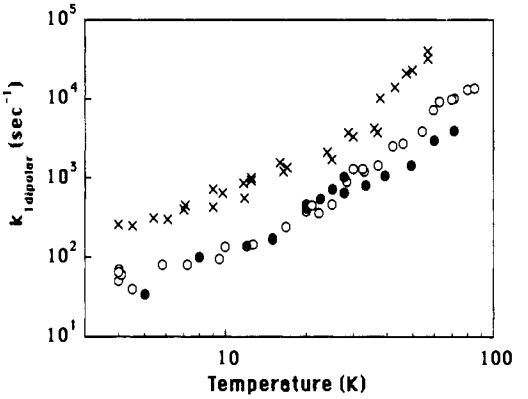

Figure 1. Dipolar spin-lattice relaxation rate constants ($k_{1dipolar}$) for $Y_D^{\bullet}$ obtained as a function of sample temperature in Mn-depleted PSII membranes (●) and in O_2-evolving PSII membranes in the S_1 active state (×) or in the S_1 resting state (○). Each data point is the average of three or four measurements with standard deviations typically 10-20% of the average value.

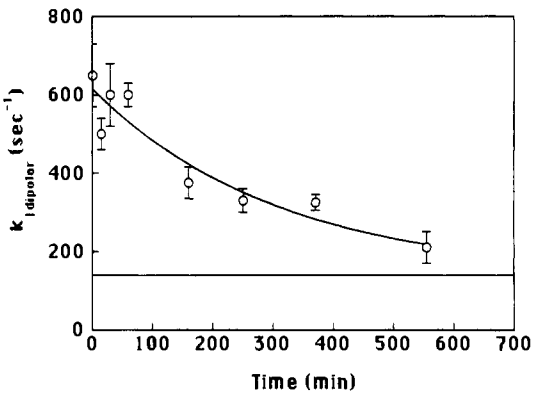

Figure 2. Change of the dipolar spin-lattice relaxation rate constant for $Y_D^{\bullet}$ (measured at 12 K) as a function of dark adaptation time at 0 °C. The sample is initially in the S_1 active state, and the exponential decrease of $k_{1dipolar}$ to its S_1 resting state value ($k_{1dipolar} = 140\ s^{-1}$) takes place with a half-time of 3.5 ± 0.7 h. The solid horizontal line is drawn at the value of $k_{1dipolar}$ at 12 K in the S_1 resting state.

In contrast, the dipolar spin-lattice relaxation rate constants of $Y_D^{\bullet}$ in S_1 resting state samples are indistinguishable from those of Mn-depleted PSII membranes below 30 K. This result shows that the ground spin state of the Mn cluster in the S_1 resting state does not contribute a relaxation enhancement pathway more efficient than that provided by the non-heme Fe(II). The fact that the dipolar rate constant increases with temperature shows that the system is in the slow relaxation limit, as discussed by Hirsh et al.[5] Previous results[7,8] show that, in this limit, even the slowly-relaxing $S = {}^1/_2$ multiline EPR signal form of the S_2 state enhances the spin-lattice relaxation of $Y_D^{\bullet}$. Therefore, we conclude that the ground spin state of the Mn cluster in the S_1 resting state is diamagnetic.

In both the S_1 active and resting states, a break is observed in the temperature dependence of the dipolar rate constants at around 30 K. The steeper temperature dependence for $T > 30$ K is probably an indication of a higher spin state of the Mn cluster being populated[3] and causing an additional contribution to the spin-lattice relaxation kinetics of $Y_D^{\bullet}$.

One can follow the change of the Mn cluster from the short dark-adapted state to the diamagnetic S_1 resting state. The dipolar rate constants were determined after successive dark incubations

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