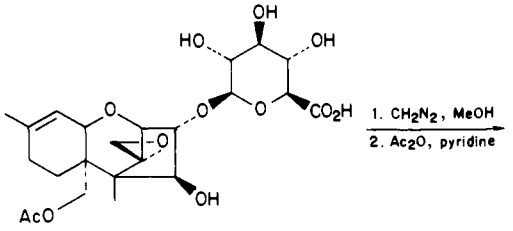
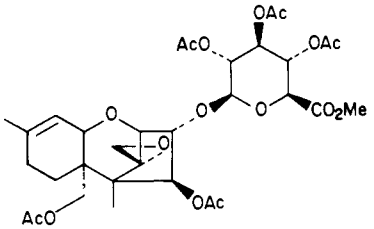
The FAB mass spectrum[18] of **3** is consistent with a 1:1 adduct of a monoacetoxyscirpendiol and glucuronic acid [*m/e* 501 (MH^+), 307 ($M^+ - C_6H_9O_7$), 265 ($M^+ - C_6H_9O_7 - C_2H_2O$)], a conclusion supported by ^1H NMR data which showed a single acetyl resonance.[17] Comparison of the ^1H signals for H-4 (δ 4.43), H-3 (4.43), and H-15 (4.20 and 3.88) of **3** measured in MeOH-d_4 with those of **4** (δ 4.21, H-4; 4.06, H-3; 4.15 and 3.91, H-15) and **5** (δ 5.54, H-4; 4.19, H-3; 3.93 and 3.45, H-15) suggested that **3** is a glucuronide derivative of **4**. This assignment was verified by incubation of [^3H]-**3** (3.7 μM) with limpet β-glucuronidase (40 units, 200 units/mL) in 0.2 M NaOAc (pH 4.5) for 18 h (37 °C) which afforded **4** in 73% yield (HPLC analysis).[19] The linkage between the trichothecene nucleus and glucuronic acid was determined following conversion to the peracetate methyl ester derivative **6**.[20] This compound proved to be identical with an

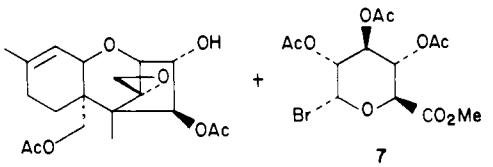


3



6

Ag2CO3
C6H6-CH2Cl2,
25-30%
(85-90% based on recovered anguidine)



1 **7**

authentic sample synthesized from anguidine (**1**) and bromo sugar **7** by a Koenigs–Knorr reaction (25–30% yield; 85–90% based on recovered anguidine).[21] These experiments rigorously establish that the glucuronic acid residue is attached to C3–OH of **4** and that the glycosidic linkage is β.

A combination of in vitro and in vivo studies are necessary to fully understand the pathways of foreign compound metabolism. In vitro studies are particularly useful in defining the types of transformations expected in vivo, thereby facilitating structural characterization of in vivo metabolites. For example, evidence from a number of laboratories has pointed toward the production and excretion of very polar trichothecene metabolites.[5e,g,h] On the basis of our work it is now reasonable to speculate that one or more of these as yet unidentified compounds may be glucuronides. Indeed, we have found that mice treated with [^3H]-anguidine excrete most of the dose in urine and that one of the major urinary metabolites is a glucuronide (substrate for limpet β-glucuronidase).[22] This, together with our finding that **3** is a poor inhibitor of protein synthesis relative to anguidine,[9] highlights the need to fully understand the nature and scope of metabolic transformations responsible for trichothecene modifications since these processes undoubtedly influence the biological half-life and the observed toxicity. Such studies are in progress and will be reported in due course.

Acknowledgment. This research was supported by the U.S. Army Medical Research and Development Command (Contract DAMD 17-82-C-2235).

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(19) Glucuronide **3** is not a substrate for bovine liver or *E. coli* β-glucuronidases.

(20) Data for **6**: mp 94.5–97 °C (CH_2Cl_2); $[\alpha]^{23}_D$ – 11° (*c* 1.61, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 5.71 (d, 1 H, *J* = 2.8 Hz, H-4), 5.47 (br d, 1 H, *J* = 5.3 Hz, H-10), 5.25 (m, 2 H, H-3′ and H-4′), 5.09 (dd, 1 H, *J* = 7.7, 9.2 Hz, H-2′), 4.76 (d, 1 H, *J* = 7.5 Hz, H-1′), 4.35 (dd, 1 H, *J* = 2.9, 4.9 Hz, H-3), 4.14 and 4.02 (AB, 2 H, *J* = 12.3 Hz, H-15), 3.96 (m, 2 H, H-5′ and H-11), 3.74 (d, 1 H, partially obscured by CO_2Me, H-2), 3.71 (s, 3 H, CO_2Me), 3.05 and 2.75 (AB, 2 H, *J* = 3.9 Hz, H-13), 2.11–2.00 (five s, 15 H, OAc), 1.68 (s, 3 H, H-16), 0.72 (s, 3 H, H-14); IR ($CHCl_3$) 2980, 2950, 2930, 2910, 1750 (br), 1440, 1370, 1250–1170 (br), 1070–1020 (br) cm^{-1}; mass spectrum (FAB, glycerol dispersion), *m/e* 682 (M^+), 622 (M^+ – HOAc); EI mass spectrum, *m/e* 622 (M^+ – HOAc). Anal. Calcd for $C_{32}H_{42}O_{16}$: C, 56.28; H, 6.20. Found: C, 56.10; H, 6.37.

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(22) The FAB mass spectrum (glycerol dispersion) of this metabolite shows a molecular ion at *m/e* 523 (MNa^+) and the product of β-glucuronidase treatment is monoacetate **4** (HPLC analysis). However, HPLC coelution studies have shown that this metabolite is not **3**. A rigorous structure proof which will establish the position of glucuronidation (apparently at C4) is in progress.

Picosecond Time-Resolved Raman Studies of Photodissociated Carboxymyoglobin

Eric W. Findsen,† Thomas W. Scott, Mark R. Chance, and Joel M. Friedman*

AT&T Bell Laboratories
Murray Hill, New Jersey 07974

Mark R. Ondrias*

Department of Chemistry, University of New Mexico
Albuquerque, New Mexico 87131

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X-ray crystallographic studies of myoglobin reveal that in liganded Mb the protein environment about the heme is different from that of the equilibrium deoxy species.[1a,b] These structural changes are qualitatively similar to the more exaggerated changes observed in X-ray crystallographic comparisons between deoxy and carboxy hemoglobin.[2] The structural changes in the heme environment induced by ligand binding can be studied using time-resolved resonance Raman spectroscopy.[3] Transient absorption studies[4–6] have shown that within a fraction of a picosecond subsequent to ligand photolysis from hemoglobin and myoglobin the heme absorption spectrum qualitatively resembles that of equilibrium deoxyheme. Protein reorganization in response to changes at the heme is expected to occur more slowly. Na-

† Present address: Department of Chemistry, University of New Mexico, Albuquerque, NM 87131.

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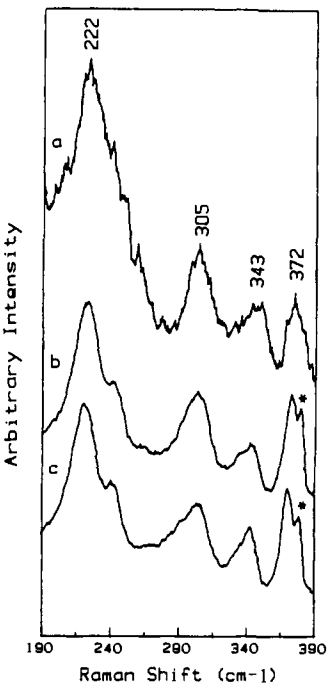

Figure 1. Low-frequency spectra of Mb obtained with Soret excitation: (a) COMb photolyzed with 20–30 ps pulses, (b) COMb photolyzed with 10-ns pulses, (c) deoxy-Mb, 10-ns, pulses. Trace (a) was produced using 20–30-ps pulses of ~435 nm from the 10-Hz output of the YAG laser system (average energy 1.0–1.5 mJ/pulse). The sample was buffered to pH 7.5 in 0.1 M Tris. Spectral resolution was ~10 cm^{-1} and the spectrum is the unsmoothed sum of three scans. Absorption spectra were recorded before and after the Raman experiment. Conditions for traces (b) and (c) were similar to those in ref 7. Asterisks denote Raman lines of the sapphire cells used in the nanosecond experiments.

nosecond and longer time-resolved Raman studies of photodissociated hemoglobins reveal the influence of the as yet unrelaxed liganded protein structure upon the deoxyheme.[7-11] By comparing equilibrium and nonequilibrium (photodissociated) forms of deoxy-Hb for both the R and T quaternary structures of hemoglobin, it is possible to isolate the structural changes that arise exclusively from ligand binding induced differences in the tertiary structure of the local heme pocket and not from a change in quaternary structure. In contrast to Hb, Mb does not exhibit differences in the nanosecond spectra of the photolyzed COMb compared to stable deoxy-Mb.[3,8] This result indicates that with respect to perturbations of the heme, the protein structure of Mb does not retain memory of ligation over a 10-ns interval subsequent to dissociation. In this note we extend these measurements into the picosecond regime in order to better understand the structural dynamics within the heme pocket of Mb and its associated implications for reactivity.

Picosecond time-resolved resonance Raman spectra of photodissociated COMb were generated by using blue laser pulses (~435 nm) at 10 Hz to both completely photolyze as well as probe the sample. The blue pulses were generated by anti-Stokes Raman shifting (hydrogen gas cell at 300 psi) single pulses of the second harmonic of an active–passive mode-locked Nd-YAG laser (Quantel). A 250 mm focal length lens focused the pulses (1-1.5 mJ/pulse) onto a flowing stream of recirculating COMb (~2×10^{-4} M) cooled to ~12 °C. The scattered light was collected at 90° to the laser excitation beam and dispersed through an instruments SA double monochromator equipped with a cooled phototube detector. The output of the detector was fed to a Princeton Applied Research Model 162 boxcar integrator system. The output of the boxcar was then stored in a signal averager which allowed accumulation of several scans to increase the signal to noise. A slowly scanned optical delay line was used to measure the rise time of a subpicosecond absorption change and gave an effective autocorrelation time (pulse width) of 20–30 ps for the blue pulses.

Figure 1 shows the low-frequency resonance Raman spectra of deoxy-Mb and COMb generated using either 10-ns or 20–30-ps pulses. The absence in all cases of a 1375-cm^{-1} peak in the high-frequency (not shown) region of the spectra indicates that photolysis is complete for the two COMb results. The picosecond spectrum represents the first such spectrum taken with blue excitation and high enough pulse energies to generate a large population of photoproduct. Moreover, the low-frequency modes (in particular the Fe–His mode at 222 cm^{-1})[17,18] are resonantly enhanced only with Soret excitation. In Figure 1 it can be seen that the peak frequencies of ν_{Fe-His} and the 343-cm^{-1} peak are invariant over the three spectra. These modes are of particular interest because both display prominent time-dependent behavior in hemoglobins subsequent to ligand photolysis. The spectra presented here indicate no memory within the Mb protein structure of the heme having been ligated within either 10 ns or 20–30 ps of photolysis. The intrinsic strength and geometry of the Fe–His mode in photolyzed Mb is established simultaneously with the movement of the Fe out of the porphyrin plane subsequent to photolysis. The slight broadening of the Fe–His mode and small differences in the modes at 343 and 372 cm^{-1} in the picosecond spectrum relative to the nanosecond spectrum may be indicative of some heterogeneity in the heme environment (such as rotational freedom about the Fe–His bond) which we cannot quantify due to the spectral resolution and signal-to-noise ratio of the low-frequency picosecond spectrum.

In contrast, similar studies of R and T state hemoglobin transients show that the large ligation induced differences observed at 10 ns[7-11,13] are also fully apparent 20–30 ps subsequent to photolysis.[12] These include a shift to higher frequency of ν_{Fe-His} and an absence of a band at ~340 cm^{-1} and persist for 10's of nanoseconds to several microseconds depending on both the species of hemoglobin and the solution conditions used. In hemoglobins the initial transient geometry is further modulated by subsequent relaxation of the proximal heme pocket resulting in the observed evolution of the Fe–His mode to a lower (i.e., steady-state) frequency. We find no evidence for any such large-scale relaxation processes in the proximal heme pocket of Mb.

The finding that within picoseconds photodissociated Mb is qualitatively indistinguishable from the stable deoxy species has implications regarding the origin of the difference in reactivity between R state HbA and Mb. The frequency of the iron–proximal histidine stretching mode, ν_{Fe-His}, has been shown to correlate with ligand binding properties. The higher the frequency the more favorable the environment for binding.[7,13] This correlation has been reported for affinity, off rates, and geminate rebinding. Mb has a lower affinity for O_2 than R state HbA[15] and the yield of geminate rebinding is reduced for Mb compared to HbA.[14] Deoxy Mb and R state deoxy HbA have approximately the same value for ν_{Fe-His} (222 ± 1 cm^{-1}). Over the psec and nsec time course for geminate rebinding, however, photodissociated HbA has a ν_{Fe-His} of ~230 cm^{-1} whereas within picoseconds of photolysis, photodissociated Mb reassumes its deoxy value of 222 cm^{-1}. The net result is that the heme in Hb is constrained by the unrelaxed proximal pocket to a geometry more nearly like that of the liganded species than is the heme in Mb. Thus, until the proximal heme pocket begins to relax, ligand rebinding in Hb

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encounters a lower energetic barrier whereas in Mb ligand rebinding energetics are instantaneously those of the steady-state system.

Indeed, measurements of ligand binding energetics using temperature-dependent transient absorption techniques have established that the final barrier to ligand binding (i.e., the barrier encountered at the heme) is much larger for Mb than Hb.[16] On the basis of recent studies of ν_{Fe-His} in a large variety of R- and T-state hemoglobins, it was concluded that the variation in ν_{Fe-His} originates primarily from a protein induced tilt of the proximal histidine (within its own plane) with respect to the heme plane.[7-9,13] An increase in the tilt, which decreases ν_{Fe-His}, increases the barrier for ligand binding by making it energetically more costly to move the iron into the heme plane and thus inhibits geminate rebinding. Given the above correlation and the lower value of ν_{Fe-His} in the transient Mb species over the time course of geminate rebinding relative to Hb transients, it is possible that the reduced geminate yield,[14] the increased off rates, and the net lower affinity of Mb relative to R-state HbA[15] do not result from differences in the average steady-state tertiary structures about the heme but arise primarily from differences in the dynamics of heme–protein interactions in these proteins.

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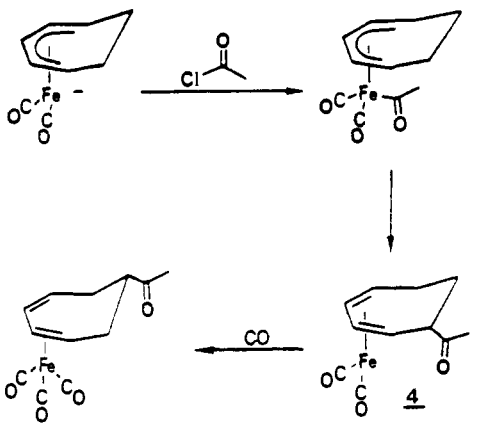
Acylation of Dienyliron Anions. Selective Formation of Exo or Endo Acyl Substituted Iron Diene Complexes

Gregory M. Williams* and Duane E. Rudisill

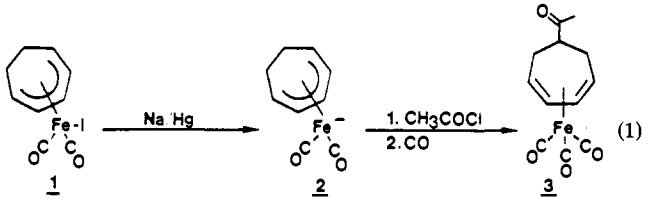
Department of Chemistry
University of California, Irvine
Irvine, California 92717
Received November 5, 1984

(Diene)iron Tricarbonyl complexes are among the more successful stoichiometric organotransition-metal reagents developed for organic synthesis. As their cationic dienyl salts, $Fe(CO)_3$ dienes react with a wide array of nucleophiles at the terminal carbon of the pentadienyl ligand, exo to the metal center.[1] In contrast, the complementary route, functionalization of iron dienes by *electrophiles*, has seen only limited development.[2-4] In this paper we report that anions derived from tricarbonyl(cycloheptatriene)iron react with acetyl chloride to provide acyl-substituted tricarbonyliron dienes. Furthermore, by controlling reaction conditions it is possible to change the nature of the iron anions such that *either* exo or endo (Acdiene)$Fe(CO)_3$ complex can be formed as the major product.

Scheme I



The conversion of (η^5-dienyl)iron tricarbonyl cations to their neutral iodo analogues is accomplished by reaction with potassium iodide.[5] The cycloheptadienyliodoiron species **1** is known to undergo rapid one-electron reduction to produce a metal–metal bonded dimer which is isoelectronic with $[(C_5H_5)Fe(CO)_2]_2$. We find that prolonged reaction between **1** and Na/Hg (THF, 7% HMPA, 25 °C, 4 h) produces a new species which we suggest is the anion (η^5-C_7H_9)$Fe(CO)_2$ (**2**).[6] Anion **2** reacts with acetyl chloride (1.3 equiv); subsequent treatment with CO (1 atm, 24 h) provides an acyl-substituted (diene)$Fe(CO)_3$ complex **3** (eq 1). Species **3** can be isolated in 45% yield as air-stable yellow



(1)

crystals. An X-ray diffraction study shows that **3** is a *symmetrical* iron diene with the acetyl group on the endo face of the coordinated diene. Proton and carbon NMR are in full accord with the proposed structure.[7]

We suggest that initial acylation of **2** occurs at the metal center to give an (η^1-acetyl)iron intermediate (Scheme I). The acyl group undergoes reductive migration to the terminal carbon of the pentadienyl ligand to provide C-5 endo-substituted (acyldiene)iron **4**. Migration of the $Fe(CO)_3$ fragment, presumably through an allyliron hydride intermediate, places the *endo*-acetyl at C-6. Steric effects could account for the isomerization of **4** to **3**. Little is known about the conformation of coordinated cycloheptadienes; however, for **3**, the ring adopts a boat conformation. Furthermore, Pearson has argued (on the basis of ^1H NMR data) that (5-*exo*-C_7H_9X)$Fe(CO)_2(PR_3)$ species also prefer the boat conformation and that boat–chair interconversion should be facile.[8] If this is also the case for 5-endo-substituted species such as **3**, then steric interaction between acetyl and $Fe(CO)_3$ in the boat conformation could drive the isomerization.

Three other products have been isolated from the reaction shown in eq 1. Two of these, (cycloheptadiene)iron tricarbonyl and the known exo–exo carbon–carbon bound dimer of $(C_7H_{10})Fe(CO)_3$ probably arise from one-electron reduction of **1**. These two products are likely derived from radical intermediates which react

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