Table I. Rate Constants for the Decomposition of 1 and 2 at 60.00 ± 0.02 °C

diazene	solvent[a]	10^6k, s^{-1} [b]	$k_{meso}/k_{(\pm)}$
meso-2	C_6H_5Cl	15.5 ± 0.6	1.1
(±)-2	C_6H_5Cl	14.0 ± 1.4	
meso-1	C_6H_5Cl	21.1 ± 0.4	1.5
(±)-1	C_6H_5Cl	14.5 ± 0.1	
meso-1	DPPC	35.5 ± 0.5	6.2
(±)-1	DPPC	5.7 ± 0.2	

[a] Diazenes were typically 0.002 M in chlorobenzene and 5–10 mol % in DPPC. [b] The rates presented are weighted means from N_2 evolution and HPLC analysis and are given ± the standard error of linear regression analysis.

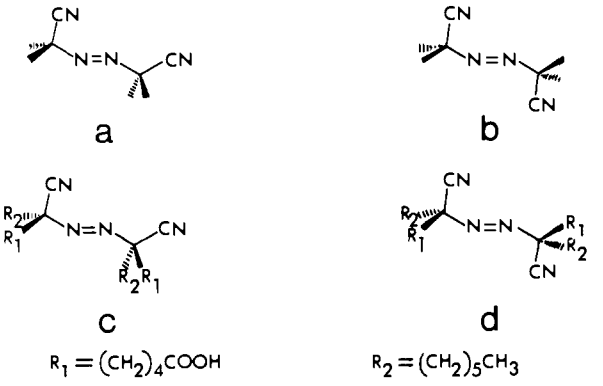

Figure 1. Conformation of diazenes: (a) AIBN in non-*S* conformation; (b) AIBN in *S* conformation; (c) *meso*-**1** in non-*S* conformation; (d) (±)-**1** in *S* conformation.

850/150/2) was assigned the (±) structure by resolution with (−)-quinine.

Decomposition of *meso*- and (±)-**1** in chlorobenzene (0.002 M) shows some rate differentiation, the meso compound decomposing 1.5 times faster than the (±) diastereomer (Table I). The greatest diastereomeric kinetic differentiation, however, is seen when **1** is decomposed in aqueous emulsions (pH 7) of DPPC (as multilamellar vesicles), a rate ratio of over 6 being observed. In a typical experiment, DPPC (15 mM) and freshly purified **1** (0.75 mM) in pH 7 phosphate buffer were vortexed to give a milky emulsion of MLVs. Nitrogen evolution was then measured by a pressure transducer,[8] or if HPLC was used to monitor the reaction, 0.90 mM lauric acid was added to the MLVs as internal standard and the decomposition was monitored by sampling from a vessel open to the atmosphere.

One rationale for the kinetic differentiation observed[9] is the possibility that the organizational properties of the lipid bilayer influence the conformation of ground-state diazene or transition state of decomposition. Diazenes similar to **1** such as AIBN have been studied by single-crystal X-ray analysis.[10] The nitriles, diazene, and connecting quarternary carbons all lie in a plane in what is termed the *S* conformation (Figure 1). As shown in the figure, the (±)-diazene can adopt the *S* conformation and place both hydrophilic carboxylate chains on one side of the diazene plane and both hydrophobic hexyl groups on the other side of that plane. The (±) diastereomer may thus adopt the *S* conformation and satisfy the amphipathic requirements of the lipid bilayer. This is not possible for the meso compound. It seems clear then, that the oriented medium could have a dramatic *and different* effect on ground- or transition-state geometries of the meso and (±) diastereomers and thus influence the kinetics of decomposition.

The bilayer-induced diastereomeric differentiation reported here indicates that lipid bilayer hosts may have a significant effect on the course of reactions involving diastereomeric reactants and transition states. Bilayer-induced selectivity in the formation of diastereomeric products would also appear to be possible. These aspects of bilayer-induced stereodifferentiation are currently under investigation in our laboratories.

Supplementary Material Available: A discussion of low-angle X-ray analysis of **1** incorporated into DPPC along with a figure showing a bilayer electron density map is presented (2 pages). Ordering information is given on any current masthead page.

(8) We thank Drs. G. Burton and K. Ingold for their design of a pressure measuring transducer.

(9) The kinetic differentiation of diastereomers we observe in chlorobenzene is greater than has been observed for other diastereomeric diazenes. See, for example: (a) Overberger, C. G.; Berenbaum, M. B. *J. Am. Chem. Soc.* **1951**, *73*, 2618–2621. (b) Scheppele, S. E.; Seltzer, S. *Ibid.* **1968**, *90*, 358–362. (c) Overberger, C. G.; Labianca, D. A. *J. Org. Chem.* **1970**, *35*, 1762–1770.

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Proton NMR Study of the Mechanism of the Heme–Apoprotein Reaction for Myoglobin

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Myoglobin and hemoglobin are globular proteins which noncovalently enfold protoheme IX. Considerable effort has been extended toward defining their single-crystal structures[1–4] whose unique conformations are the basis for the interpretation of functional studies. The rates of the reversible reaction between protoheme IX (E in Figure 1) and apomyoglobin and apohemoglobin have been studied by optical spectroscopy[5–7] which led to the currently accepted mechanism whereby both apoproteins combine with protoheme IX to yield a single, native conformation and this reaction is complete within a time scale of a millisecond.[8,9] The complete understanding of this reaction is not only highly relevant to the biosynthesis of b-type hemoproteins[10] but also has important ramifications for the interpretation[11–14] of physicochemical measurements made on reconstituted proteins even when using native protoheme IX.

We report herein a real time ^1H NMR analysis of the reconstitution of myoglobin whose results are in essential variance with both the presently accepted mechanism of the reconstitution as well as the nature of the conformation of the native protein. Sperm whale myoglobin was reconstituted[15,16] in situ within a NMR tube by mixing equivalent amounts of protohemin-IX or reduced[17] protoheme IX–CO with apomyoglobin, and the time course of the reaction was followed by ^1H NMR. A in Figure 1 shows that the spectrum immediately after reconstitution consists of two sets of heme resonances: the set X_i arising from "native" metMbH_2O previously assigned by isotope labeling[18] and a set Y_i, whose

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(17) The reduced protoheme IX–CO complex was also used to eliminate possible effects of aggregation, as discussed previously.[5,6]

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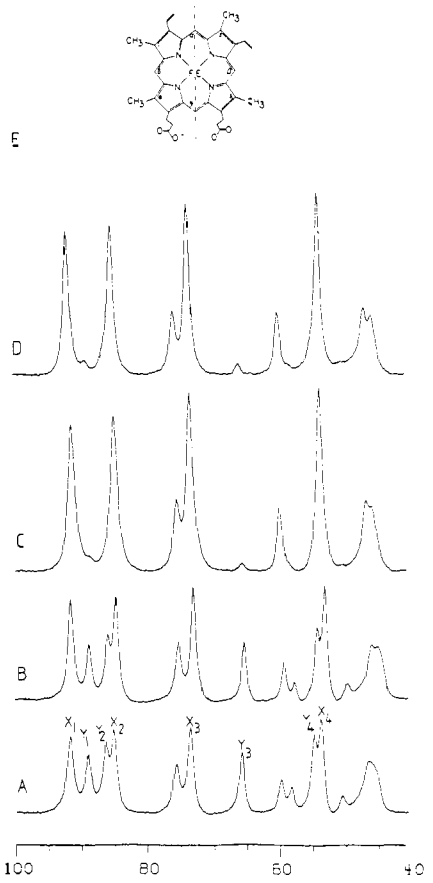

Figure 1. The downfield portion of the 360-MHz ^{1}H NMR spectra of metMbH_2O 25 °C at various time intervals after reconstitution is shown: (A) 8 min, (B) 140 min, (C) 800 min (pH 5.7), (D) native protein (pH 6.1). The structure of protoheme IX is shown in E: In situ reconstitution of metMbH_2O was carried out by adding an equivalent amount of 0.01 M NaO^2H solution of hemin to 0.5 mL of apoMb in 0.1 M phosphate buffer, pH 7.0. The pH was immediately adjusted with 0.1 M ^{2}HCl or 0.1 M NaO^2H to the desired pH value. Initial and final pH readings were then made and were consistent.

comparable initial intensity decreases with time. The spectrum in C exhibits no further intensity changes with time and is essentially identical with that obtained for the purified native metMbH_2O, as found in D. The traces of the ring-current shifted region of the ^{1}H NMR spectrum of the reconstituted MbCO[19] are given in Figure 2. Again two resonances, X_1 and Y_1, are seen immediately after reconstitution (A), with the area of X_1 increasing with time at the expense of Y_1, until a spectrum indistinguishable from that of native MbCO (B) is obtained.[20]

Qualitative conclusions that immediately follow are that (1) reconstitution yields a heterogeneous protein system consisting of comparable amounts of two components, only one of which corresponds to the "native" protein, (2) the "intermediate" component converts to the "native" conformation on a time scale $\sim 10^7$ more slowly than that reported[5-7] for the complete reconstitution of Mb for the same systems, and (3) the "intermediate" form is present both at equilibrium and in the "native" protein, indicating that even the native protein is conformationally heterogeneous.

Two sets of resonances are observed when reconstituting with any heme IX derivative, indicating that this intermediate is quite

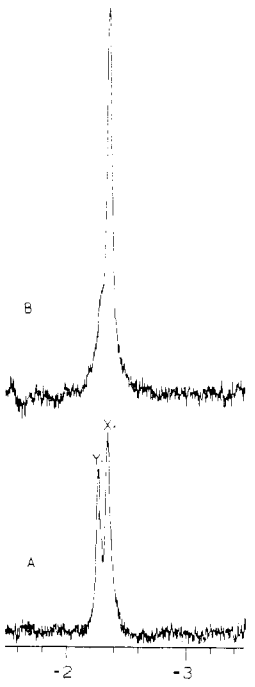

Figure 2. The ring-current shifted portion of 360-MHz ^{1}H NMR spectra of MbCO, 25 °C, at various time intervals after reconstitution is shown: (A) 15 min, (B) 2 weeks (at equilibrium) (pH 7.6).

general. However, when a heme is used that possesses true rotational symmetry about the α-γ-meso axis (protoheme XIII, 2,4-dimethyldeuteroheme IX), only a single set of resonances is observed at all times. This argues that the intermediate is the holoprotein with the heme rotated 180° about the α-γ-meso axis from that found in the crystal.[21] Indeed, detailed isotope labeling experiments of the two components at equilibrium support this structure for the intermediate.[22]

The structure of apoMb is though to be similar to that of Mb but with reduced helical content and rigidity.[23] The initial formation of comparable amounts of Mb with the two heme orientations dictates that the formation of the iron histidine bond does not discriminate between the two sides of the heme and that the permutational asymmetry of vinyls and methyls does not become important until the final stages of the relaxation of the polypeptide conformation to that of the holoprotein. Thus the intermediate is a kinetically trapped, metastable form of the protein. The equilibration requires the rupture of the axial bond and hence must involve at least partial, transient unfolding of the protein.[24] The slow equilibration under conditions precisely those employed in the standard literature method[16] for reconstituting b-type hemoprotein brings into question the nature of the samples on which previous measurements have been made.[11-14] Moreover, this very slow equilibration rate (10^{-5} s^{-1}) for the heme reorientation in the physiologically relevant pH range suggests that either the enfolding of the heme by the apoprotein is enzyme mediated to yield only the "native" form or that the dichotomous heme orientations in Mb are functionally relevant[25] in vivo.

Current work is directed toward further characterizing the dynamics, thermodynamics, and functional properties of the heme

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(25) Preliminary data on Mb samples containing different ratios of the two heme orientations indicate a higher oxygen affinity for the "wrong" heme orientation, suggesting that even properties for the "native" protein may have to be reinterpreted on the basis of a 12:1 ratio of the two heme orientations observed at equilibrium (Livingston, D. J.; Davis, N. L.; La Mar, G. N.; Brown, W. D., unpublished results).

disorder in native and reconstituted hemoproteins.

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Chemoselective Desilylation of Silyl Enol Ethers with Tributyltin Fluoride Catalyzed by Palladium

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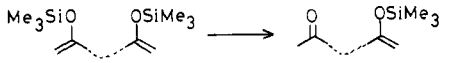
Differentiation of more than two types of ketone functions is often required in organic synthesis. For years, selective protections (e.g., ketalization or reduction) have been conventionally used as exemplified in various natural product syntheses. Considering that silyl enol ethers have found widespread use in synthetic organic chemistry,[1] selective silylation of a certain ketone group of diketo compounds may provide another useful tool for this purpose. Although many methods have been developed for ketone silylation, only a few examples are reported for selective monosilylation of diketones that rely on differences in kinetic acidities.[2] We wish to describe here an alternative approach based on selective desilylation of bis(silyl enol ethers) that could be prepared readily by a variety of methods.

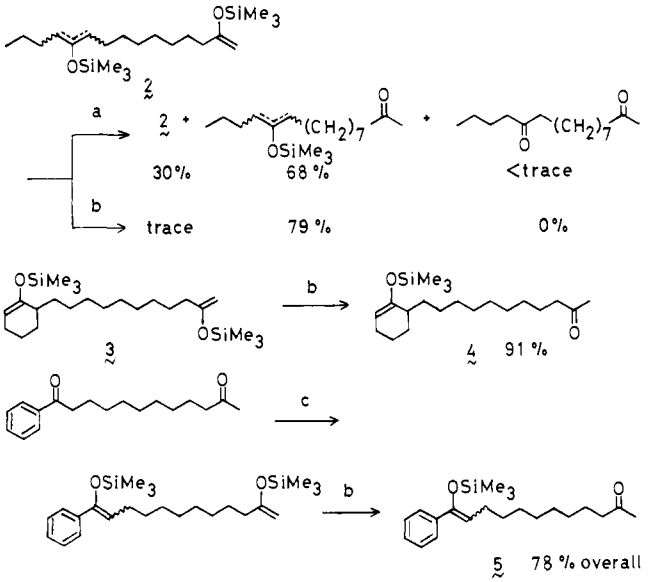


(1)

We have found that tributyltin fluoride[3] removes the silyl group from silyl enol ethers quite selectively under the influence of a palladium catalyst. As the catalyst, $PdCl_2(P(o\text{-}MeC_6H_4)_3)_2$ has proved to be the most effective.[4] Although the reaction proceeds in the absence of the catalyst, the catalyst accelerates the desilylation dramatically to complete the process within a reasonable period. The efficacy of the catalyst has been demonstrated by the following experiments. 5-Methyl-2-(trimethylsiloxy)-1-hexene (**1**) was treated with Bu_3SnF (1.05 equiv) and a palladium catalyst (3 mol %) in refluxing benzene for 30 min. After quenching the reaction mixture with wet acetone, isopentyl methyl ketone (and **1**) was recovered in 19 (80)% (without a catalyst); 21 (78)% ($Pd(PPh_3)_4$); 28 (67)% ($PdCl_2(CH_3CN)_2$); 31 (52)% ($PdCl_2(PPh_3)_2$); 96 (2)% ($PdCl_2(P(o\text{-}MeC_6H_4)_3)_2$).

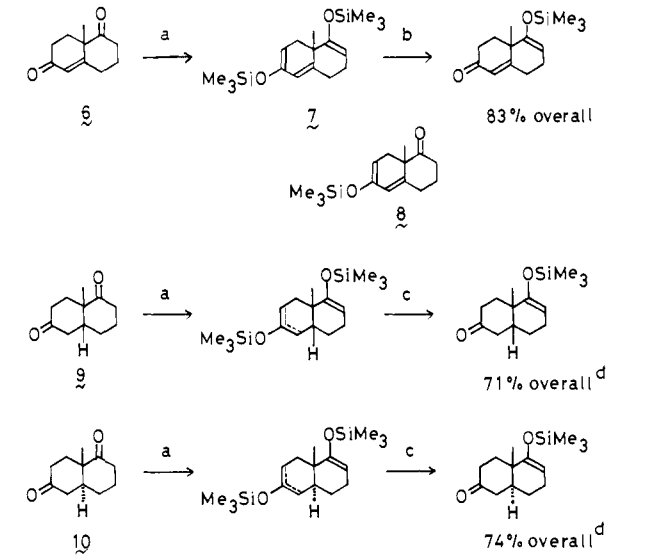
Of particular interest, the reaction rate was highly dependent on a steric congestion around the double bond of the silyl enol ether: less hindered cases undergo desilylation much more rapidly than more hindered ones. Thus, silyl enol ethers of methyl ketones could be desilylated under standard conditions as above where other hindered silyl enol ethers remained unattacked. However, the observed selectivity may not be due to the Pd catalyst, but intrinsically due to Bu_3SnF as evidenced by running the reaction without the catalyst (cf. the first equation of Scheme I). Several results are shown in Scheme I.

Scheme I



[a] Bu_3SnF (1.05 equiv), C_6H_6, reflux, 7 h. [b] Bu_3SnF (1.05 equiv), $PdCl_2(P(o\text{-}MeC_6H_4)_3)_2$ (3 mol %), C_6H_6, reflux, 0.5 h. [c] LDA, THF, −78 °C; Me_3SiCl, −78 °C → room temperature; no purification.

Scheme II



[a] LDA, THF, −78 °C; Me_3SiCl, −78 °C → room temperature. [b] Bu_3SnF (2.5 equiv), $PdCl_2(P(o\text{-}MeC_6H_4)_3)_2$ (8 mol %), C_6H_6, reflux, 4 h. [c] Bu_3SnF (5 equiv), $PdCl_2(P(o\text{-}MeC_6H_4)_3)_2$ (15 mol %), C_6H_6, reflux, 11 h. [d] The relatively low yield is partly due to incompletion of the reaction.

In each case, after column chromatography on silica gel, the product can be isolated without any contamination of the regioisomer arising from reversed selectivity. Moreover, no formation of the parent diketone was confirmed by TLC analysis of the crude reaction mixture.[5] These facts clearly indicate the high selectivity of the present method.

Further, selective removal can also be achieved with other types of enol ethers by employing forcing conditons (e.g., use of an excess amount of Bu_3SnF and a prolonged reaction period).[6] The results

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(4) Similarly, higher efficacy of this catalyst has also been observed in the arylation of silyl enol ethers. See ref 3.

(5) Desilylation of **2** with tetrabutylammonium fluoride (1 equiv in THF at 0 °C or even at −78 °C) gave a considerable amount of the diketone.

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