spectrum of complex **2** is very similar to those of previously reported high-spin Fe(III) porphyrin complexes with a C_s symmetry such as Fe^{III}(*N*-alkylTPP) complexes.[13] The assignment of the different signals of this ^1H NMR spectrum was made by comparison of the spectrum of complex **2** with those of its analogues prepared from $TPPH_2$ deuterated either on the pyrrole ($TPPH_2$-d_8) or mesophenyl ($TPPH_2$-d_{20}) rings or from tetrakis(*p*-chlorophenyl)porphyrin. Because of the C_s symmetry, the pyrrole protons appear as four signals at −28.4, 81.7, 84.5, and 89.5 ppm (each 2H) and the mesophenyl protons at 6.85, 3.92 (each 2 H, H para), 12.7, 11.22, 10.93, 10.30 (each 2 H, H meta), and 6.10 and 4.10 ppm (each 2 H, very broad H ortho[14]). Two resonances for the *N*-Ts moiety appear at 13.71 (CH_3 + 2 Ar H) and 14.32 ppm (2 H). The great similarity of this ^1H NMR spectrum with those of Fe^{III}(*N*-alkylTPP) complexes suggests two possible structures for complex **2**: either a bridged FeIII–NTs–N or a FeIII–N–NHTs–TPP structure. Accordingly, demetalation of complex **2** by CF_3 COOH under anaerobic conditions leads to the *N*-(tosylamido)tetraphenylporphyin (**3**)[15] (Figure 1). The elemental analysis (C, H, N, S, Cl) of complex **2**, which is in perfect agreement with the Fe(TPP)(NTs)(Cl) formula corresponding to the bridged structure of Figure 1, allows one to discard a Fe^{III}(NNHTsTPP) structure corresponding to a [Fe(NNHTsTPP)(Cl)]$^+X^-$ formula with X^- being Cl^- or another counterion such as OH^-. Moreover, conductimetry measurements performed on complex **2** agree with the bridged structure but are completely inconsistent with the ionic structure (conductivity of **2** 10^{-3} M in CH_3CN almost equal to that of pure CH_3CN). Finally, the great stability of complex **2** at room temperature and its $g = 4.3$ EPR signal indicative of a rhombic symmetry are in agreement with the bridged structure but not with a Fe^{III}–N–NHTsTPP structure since [Fe^{III}(*N*-alkylTPP)(Cl)]$^+X^-$ complexes, which should be similar to [Fe^{III}(NNHTsTPP)(Cl)]$^+X^-$, were described as stable only below −40 °C and to exhibit $g = 5.66$ and 2.1 signals indicative of an axial symmetry.[13] Taken altogether these data clearly show the bridged structure of Fig. 1 for complex **2**.

Since complex **2** which derives from the insertion of the N–Ts moiety into a Fe–N bond of Fe(TPP)(Cl) was formed during reaction of PhI=NTs with alkenes catalyzed by Fe(TPP)(Cl), it was of interest to determine whether **2** was able to transfer its NTs moiety to an alkene or to catalyze aziridination by PhI=NR. Complex **2** alone in anhydrous CH_2Cl_2 containing 100 equiv of cyclooctene gave no aziridine after 20 h at 20 °C. However, when used instead of Fe(TPP)(Cl) under the conditions previously described for cyclooctene aziridination by PhI=NR, it acted as a catalyst leading to a similar yield of aziridine (17%).

Complex **2** is the first example of a nitrogen analogue of the Fe^{III}(TPP)[C=C(*p*-ClC_6H_5)$_2$](Cl) compound derived from the insertion of the C=CAr_2 moiety into a Fe—N bond of Fe(TPP)(Cl).[7] Although an intermediate Fe(III) ($S = {}^3/_2$) spin state was found for the latter,[7c] a high-spin Fe(III) state is observed for complex **2**. This is presumably related to the greater strength of the Fe–C bond relative to the Fe–N bond as previously observed from a comparison of the spin states of Fe(TPP)(NNR_2), Fe(II) ($S = 2$),[2] and Fe(TPP)(C=CAr_2), Fe(II) ($S = 0$).[3d]

From the aforementioned results, it is likely that the active intermediate formed during alkene aziridination by PhI=NTs catalyzed by Fe(TPP)(Cl), which could be formally written as a Fe^V=NTs complex, either transfers its NTs moiety to the alkene or undergoes an intramolecular isomerization leading to **2** (Figure 1). Even though complex **2** is not able to transfer its NTs moiety to alkenes, it is still active in catalyzing alkene aziridination by PhI=NTs.

Acknowledgment. We are grateful to Dr. I. Morgenstern-Badarau for EPR measurements and interpretations, to Dr. A. Gouyette and Dr. Rolando for mass spectrometry measurements, and to Dr. J. Devynck for conductimetry measurements.

Photochemical and Chemical Enzyme Catalyzed Debromination of *meso*-1,2-Dibromostilbene in Multiphase Systems

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Enzyme-catalyzed reactions in multiphase systems are of substantial interest in organic synthesis.[1] Various reactions were accomplished by using sepharose-supported enzyme systems in organic solvents.[2] Incorporation of enzymes as catalysts in photochemical reactions has been proven to be a promising synthetic tool.[3,4] Recently, we examined photosensitized electron transfer reactions in water–oil two-phase systems using amphiphilic electron acceptors.[5-7] We have shown that photoreduction of *N,N'*-dioctyl-4,4'-bipyridinium, C_8V^{2+}, in the aqueous phase results in the extraction of the reduced photoproduct, $C_8V^{+\cdot}$, into the organic phase. This photoproduct undergoes induced disproportionation (eq 1) to the two-electron charge relay *N,N'*-di-

$$2C_8V^{+\cdot} \rightleftharpoons C_8V + C_8V^{2+} \quad (1)$$

octylbipyridylidene, C_8V, due to opposite solubility properties of the disproportionation products in the two-phase system. Here we wish to report on the photochemically and chemically induced debromination of *meso*-1,2-dibromostilbene to *trans*-stilbene using enzyme-catalyzed systems supported on sepharose beads. In these processes ethanol, lactic acid, alanine, and formate act as electron donors for the debromination process.

In the photochemical systems ethanol, lactic acid, and alanine were used as electron donors. Sepharose beads were soaked in 0.35 mL of an aqueous solution (pH 8.2) that includes the sensitizer ruthenium(II) tris(bipyridine), $Ru(bpy)_3^{2+}$ (1.4×10^{-3} M), *N,N'*-dioctyl-4,4'-bipyridinium (octylviologen), C_8V^{2+} (2.8×10^{-3} M), as electron acceptor, nicotinamide adenine dinucleotide, NAD^+, (1.2×10^{-2} M), and ethanol (0.5 M), lactic acid (0.35 M), or alanine (0.5 M). The system where ethanol is used as electron donor included the enzyme alcohol dehydrogenase (E.C. 1.1.1.1 horse liver, 1.5 units), the system with lactic acid included L-lactic dehydrogenase (E.C. 1.1.1.27 rabbit muscle, 100 units), and with alanine as donor, L-alanine dehydrogenase (E.C. 1.4.1.1. *Bacillus subtilis*, 0.4 units) was introduced.[8] These orange beads were suspended in 4 mL of ethyl acetate that included *meso*-1,2-dibromostilbene (**1**), (3.2×10^{-3} M). The systems were deaerated and illuminated with visible light ($\lambda > 400$ nm). Analysis of the organic phase reveals that dibromostilbene is debrominated and *trans*-stilbene (**2**) is formed.[9] The rate of **2** formation as a function of illumination time is depicted in Figure 1, for the different donors. With ethanol as electron donor 100% conversion of **1** to **2** is accomplished. Ethanol is oxidized in the

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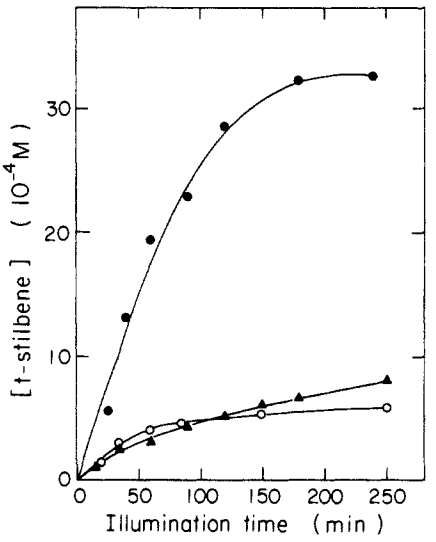

Figure 1. Rates of *trans*-stilbene formation as a function of illumination times with the different electron donors: (●) ethanol; (○) lactic acid; (▲) alanine.

Table I. Turnover Numbers (TN) of the Components in the Different Systems

donor	enzyme	TN enzyme	TN NAD^+	TN C_8V^{2+}
ethanol[a]	alcohol dehydrogenase E. C. 1.1.1.1	1900	6.3	26
lactic acid[b]	L-Lactic dehydrogenase E.C. 1.1.1.27	11400	1.3	5.5
alanine[c]	L-alanine dehydrogenase E.C. 1.4.1.1	160000	2.2	9
formate[d]	formate dehydrogenase E.C. 1.2.1.2	6800		15

[a] 100% conversion of **1** to **2**. [b] 21% conversion of **1** to **2**. [c] 35% conversion of **1** to **2**. [d] 100% conversion of **1** to **2**.

reaction to acetaldehyde while lactic acid and alanine are oxidized to pyruvic acid.[10]

The detailed mechanism leading to the debromination of dibromostilbene (**1**) has been established. Exclusion of either C_8V^{2+}, NAD^+, the electron donors, or the enzymes from the systems prevents the debromination process. This implies that all components are essential for the reaction. Exclusion of dibromostilbene (**1**) from the organic phase results upon illumination in the formation of the blue radical cation, $C_8V^{+\cdot}$, that is extracted from the sepharose beads into the organic phase. Upon stirring the system the blue color disappears and the yellowish color of the two-electron charge relay, C_8V, is produced (λ_{max} = 394 nm).[5,11] Cyclic voltametric experiments imply that the single-electron transfer product $C_8V^{+\cdot}$ is inert toward **1**, while the two-electron charge relay C_8V is the active species in the debromination process.[5] The function of NAD^+ in the systems has been established by separate experiments. We find that 1,4-dihydronicotinamide adenine dinucleotide, NADH, acts as an effective electron donor for the oxidized photoproduct $Ru(bpy)_3^{3+}$. Illumination of a homogeneous aqueous phase that includes $Ru(bpy)_3^{2+}$ (1.6×10^{-4} M) as sensitizer, C_8V^{2+} (3.3×10^{-4} M) as electron acceptor, and NADH (9×10^{-4} M) results in the formation of $C_8V^{+\cdot}$, $\Phi = 1.4 \times 10^{-2}$. These results confirm that NADH reduces $Ru(bpy)_3^{3+}$ and recycles the sensitizer.

These results allow us to suggest the cyclic mechanism outlined in Figure 2 as the route for the debromination process. In this cycle the enzymes catalyze the intermediate formation of NADH by the substrates ethanol, lactic acid or alanine. Photosensitized electron transfer results in the formation of $C_8V^{+\cdot}$ and the oxidized

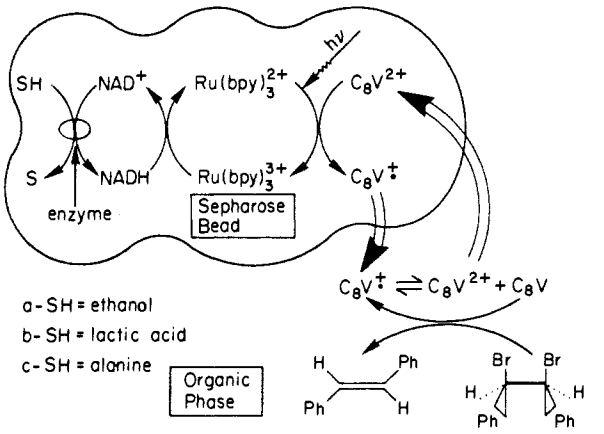

Figure 2. Cyclic photochemical debromination process mediated by NADH.

photoproduct, $Ru(bpy)_3^{3+}$, is reduced by NADH, thereby regenerating the sensitizer and NAD^+. The photoproduct, $C_8V^{+\cdot}$, due to its hydrophobic properties, is extracted from the aqueous phase into the organic medium. Disproportionation of the single-electron transfer product results in the hydrophilic oxidized from C_8V^{2+}, which is reextracted into the aqueous environment contained in the sepharose beads. Consequently, the disproportionation equilibrium is shifted and the two-electron charge relay active in the debromination process is formed.[5]

The net reactions induced by light correspond to the debromination of **1** by ethanol, lactic acid, or alanine, to yield **2** and acetaldehyde or pyruvic acid (eq 2–4). The turnover numbers

$$2CH_3CH_2OH + PhBrCH\text{-}CHBrPh \rightarrow 2CH_3CHO + t\text{-}PhHC{=}CHPh + 2HBr \quad (2)$$

$$2CH_3C(OH)HCO_2^- + PhBrCH\text{-}CHBrPh \rightarrow 2CH_3COCO_2H + t\text{-}PhHC{=}CHPh + 2Br^- \quad (3)$$

$$2CH_3C(NH_2)HCO_2H + 2H_2O + PhBrCH\text{-}CHBrPh \rightarrow 2CH_3COCO_2H + 2NH_4^+ + t\text{-}PhHC{=}CHPh + 2Br^- \quad (4)$$

of NAD^+, C_8V^{2+}, and the enzymes are summarized in Table I. It can be seen that for the system where thanol is used as electron donor the ingredients are effectively recycled without any observable deactivation. In turn, the systems where lactic acid and alanine are included are deactivated after 21% and 35% conversion of **1**, presumably due to enzyme deactivation. The common photoregeneration of $C_8V^{+\cdot}$ can be accomplished by the use of sacrificial electron donor such as EDTA or triethanolamine. In this system the donors are oxidized into fragments. In the present system, the application of donors, not active themselves in the photochemical cycle, is possible by the intermediate regeneration of NADH in the presence of the corresponding enzymes. The formed NADH exhibits electron donor properties, toward the oxidized photoproduct, $Ru(bpy)_3^{3+}$, and allows the oxidation of the alcohol, alanine, and lactic acid.

The photochemical system supported on the beads could be substituted by a chemical system generating $C_8V^{+\cdot}$. It is well established that *N,N'*-dialkyl-4,4'-bipyridinium salts (viologens) act as substrate for the enzyme formate dehydrogenase.[12] Thus, the direct reduction of C_8V^{2+} by formate could be envisaged. The beads were swollen with an aqueous solution (pH 7.0) that includes sodium formate (5 dehydrogenase.[12] 10^{-1} M) as electron donor, C_8V^{2+} (5×10^{-3} M), and the enzyme formate dehydrogenase (E.C. 1.2.1.2 *Pseudomonas oxalaticus*, 1 unit). These beads were introduced into an ethylacetate solution of **1** (3×10^{-3} M). The system was stirred under an inert atmosphere and stilbene was quantitatively formed in the organic phase. In this system formate dehydrogenase catalyzes the reduction of C_8V^{2+}. The resulting reduced product, $C_8V^{+\cdot}$, is extracted into the organic phase, where

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induced disproportionation proceeds to yield C_8V. The latter product mediates the debromination process. The next reaction corresponds to the debromination of **1** by formate (eq 5). The turnover numbers of this process are also summarized in Table I.

$$HCO_2^- + PhBrCH\text{-}CHBrPh \rightarrow CO_2 + t\text{-}PhHC{=}CHPh + 2Br^- + H^+ \quad (5)$$

In conclusion, we have demonstrated the novel application of enzyme-catalyzed photochemical reactions in multiphase systems. The photosensitized electron transfer process provides a means to regenerate NAD^+ from NADH. The proper hydrophilic–hydrophobic balance of the redox couple $C_8V^{2+}/C_8V^{+\cdot}$ allows the formation of the two-electron charge relays in the organic phase and its subsequent utilization in chemical reactions. The stability of the photochemical system for the debromination of **1**, where ethanol is an ultimate electron donor, might find a synthetic merit.

Acknowledgment. The support of the Belfer Center for Energy Research, Israel, is gratefully acknowledged.

Deuterium/Protium Fractionation Factors for Polyfunctional Organic Molecules: Direct Determination by Carbon-13 NMR Spectroscopy†

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Received September 16, 1985

The isotope exchange processes between water and readily exchangeable groups (e.g., OH, NH, and SH) are of interest in studying reaction mechanisms and elucidating the structural dynamics of biological macromolecules.[1-3] A knowledge of the equilibrium partioning of deuterium and protium between the different types of exchangeable hydrogens is needed for a detailed interpretation of kinetic and equilibrium data and for the understanding of these processes.[1-3] Jarret and Saunders have eloquently summarized the limitations of existing methods for the determination of deuterium/protium fractionation factors.[4] They have outlined an NMR method for obtaining fractionation data at multiple sites in *rapidly exchanging* systems in an aqueous medium. Their approach is based on the isotope effects on carbon-13 chemical shifts[5] and involves the measurement of chemical shifts between solutions (contained in concentric tubes) in H_2O and D_2O and between solutions in H_2O and H_2O/D_2O mixtures.[4] This paper describes a method for the direct determination of fractionation factors from the integrated intensities of the carbon-13 resonances of deuterio and protio species under conditions of *slow chemical exchange*.

The substitution of deuterium for protium leads usually to an upfield shift in the carbon-13 resonances of atoms in the vicinity of the exchangeable site.[5] Under conditions of slow hydrogen exchange relative to the magnitude of this isotope effect, separate resonances are observed for the individual isotopomers. For amides[6,7] and ammonium derivatives[8] such isotopic multiplets can be observed in an aqueous medium. Non-hydroxylic organic solvents, e.g., Me_2SO, have been employed to achieve conditions of slow exchange for other functional groups.[9-12] The carbon-13 resonance of an atom in the vicinity of a partially deuterated group containing one exchangeable hydrogen atom is a doublet, the components of which correspond to the XH and XD species. Higher multiplets are observed when several equivalent exchangeable hydrogens are present.[7,8,11] The relative intensity of the mth component in a multiplet due to n equivalent isotope effects is given by

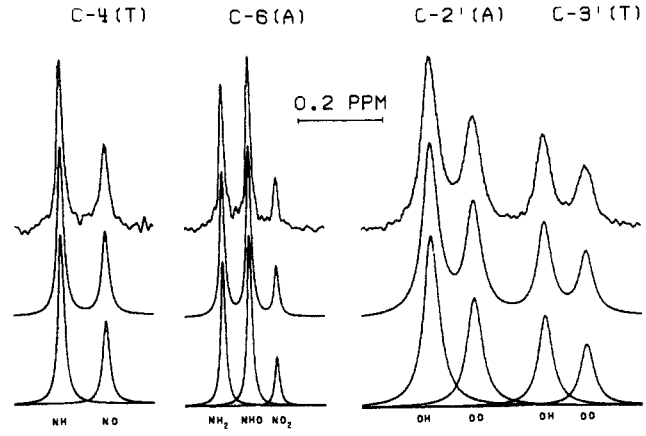

Figure 1. Isotopic multiplets for some of the carbon-13 resonances of adenosine and thymidine in their 2:1 mixture in Me_2SO-d_6: top, experimental; middle, calculated; bottom, individual components. The isotopic species are indicated under the peaks. Deuterium was introduced as CH_3OD. At equilibrium the deuterium/protium ratio as obtained from the relative areas of the CH_3OH and CH_3OD peaks was 0.71.

$$\frac{n!R^{m-1}}{(m-1)!(n-m+1)!} \quad (1)$$

where R is the deuterium/protium ratio and the first component is the protio form.[11] Thus, from the integrated intensities of the multiplet components one can obtain the deuterium/protium ratio at a given molecular site. If R_{water} is known, e.g., in dilute solutions in D_2O/H_2O mixtures of known composition, the fractionation factor

$$\alpha = R_{substance}/R_{water} \quad (2)$$

can be readily determined. In nonaqueous solvents methanol can be used as a reference compound and $R_{methanol}(R_{ref})$ measured from the components of the methanol doublet in the carbon-13 NMR spectrum. The fractionation factor for methanol has been accurately determined (α = 1.12 at 25 °C)[1] and can be used to obtain the fractionation factor of interest:

$$\alpha = \alpha_{ref}R_{substance}/R_{ref} \quad (3)$$

In this work a Nicolet 360 WB spectrometer was employed operating at 90.56 MHz for carbon-13. Accurate integrated intensities were obtained by curve-fitting of the spectral bands using the NMCCAP routine supplied by the instrument manufacturer. The rms deviation between experimental and calculated curves was usually less than 1%. An example is presented in Figure 1, where some of the resonance of thymidine and adenosine (in their mixture in Me_2SO-d_6) are shown. Deuterium was introduced as CH_3OD.

In aqueous solutions, as well as in the presence of acids or bases, the hydrogen exchange of methanol is fast. This limitation on the general applicability of the proposed method can be obviated by using N-methylacetamide ($CH_3CONHCH_3$) as the reference compound. This material is soluble in a variety of solvents and has the advantage of slow hydrogen exchange under most conditions, except for very high pH values. All three carbon-13

† Contribution No. 1861 from the Hercules Research Center.

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