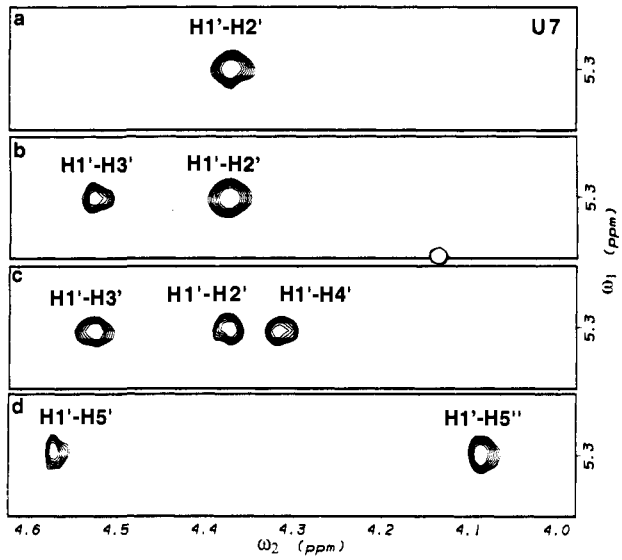

Figure 2. The H1′ (ω_1) to H2′ through H5′/H5″ (ω_2) region of the (a) HCCH-COSY, (b) HCCH-RELAY, (c) HCCH-TOCSY methylene filtered, and (d) HCCH-TOCSY methylene selected spectra on the uniformly ^{13}C labeled RNA duplex, r(GGCGCUUGCGUC)$_2$. Only the H1′ region for U7 is shown. The sample conditions were 1.8 mM RNA single strand, 150 mM NaCl, 10 mM potassium phosphate (pH = 6.8), and 0.1 mM EDTA. The labeled RNA was synthesized as previously described.[9,10,12] All the spectra were collected on a Varian VXR-500S NMR spectrometer at 30 °C.

The spectrum shown in Figure 2c is a linear combination of these two experiments, $F_1 + \alpha F_2$, where α is adjusted to cancel the 5′/5″ protons. Figure 2d is the difference of the two experiments, $F_1 - \beta F_2$, where β was adjusted to cancel the 2′ through 4′ protons. The optimal values for α and β were empirically determined by analyzing linear combinations of the first FIDs in the two experiments. An important advantage of this strategy is that, by directly selecting for only the 5′/5″ protons, it is possible to unambiguously assign the 5′/5″ protons even if these protons overlap with another proton in the same ribose ring. For larger RNAs this region of the spectrum will be more crowded, and therefore one can extend the strategy presented here to 3D heteronuclear HCCH experiments.[4-6] We have previously shown that the resolution of the RNA spectrum is substantially increased in 3D and 4D NMR experiments.[9,10]

The methods described here allow unambiguous identification of all the protons in an individual ribose ring, but each ribose ring must also be assigned to a specific residue in the RNA sequence. This assignment can be carried out by the standard sequential assignment techniques that rely on NOE connectivities between protons on neighboring residues.[1-3] However, tertiary interactions or unusual conformations in loops, bulges, or single-stranded regions of RNAs could lead to misassignments. Thus a superior method for making sequential assignments is to observe through-bond connectivities between neighboring residues by employing techniques such as the recently described hetero-TOCSY experiment.[11] We are presently testing a variety of triple-resonance (^1H, ^{31}P, ^{13}C) experiments to find optimal methods for through-bond sequential resonance assignment of uniformly ^{13}C labeled RNAs.

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Registry No. r(GGCGCUUGCGUC), 138153-87-4; ribose, 50-69-1; hydrogen ion, 12408-02-5.

Supplementary Material Available: Figure showing the H1′ to H2′–H5′/H5″ region of an HCCH-TOCSY spectrum acquired for the RNA duplex (1 page). Ordering information is given on any current masthead page.

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Non-Ionic Water-Soluble Dextran-Coupled Tetraphenylporphyrin Derivatives

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Tetraphenylporphyrin (TPP) is an important classical hemin model for a variety of biological processes.[1] However, few mechanistic studies of the reactions of TPP and its ferric derivative under biological conditions have been carried out, primarily because of the insolubility of these compounds in aqueous solutions. In attempts to overcome this problem, water-soluble ionic porphyrins have been developed.[2] However, their tendency to aggregate, as well as the tendency of their ferric derivatives irreversibly to form an inactive μ-oxo dimer, restricts their usage as a tool for physicobiochemical research.[3] Though porphyrins which cannot form a μ-oxo dimer such as picket fence porphyrins[4] have been developed, they are water-insoluble. To overcome these problems, we have designed and prepared novel non-ionic water-soluble TPP analogs, i.e., Dex-TPP and Dex-TPPFeCl (Figure 1). As we had anticipated, coupling of TPP analogs to dextran polymer made them water-soluble as well as unable to aggregate or to form a μ-oxo dimer. In this paper, preparation of Dex-TPP and Dex-TPPFeCl, their behavior in aqueous solution, and their function as represented by DNA-cleavage ability are described.

The ligand, 5-(*p*-aminophenyl)-10,15,20-tri-*p*-tolylporphyrin, and its ferric derivative (TPP-NH_2 and TPPFeCl-NH_2, Figure 1) were prepared as described previously.[5] TPP-NH_2 or TPP-FeCl-NH_2 was coupled with dextran (MW > 2 000 000) by the method described by Norman et al.[6] with minor modifications. Briefly, dextran was partially oxidatively cleaved by $NaIO_4$ (0.01–0.2 equiv) in acetate buffer (pH 5), and then TPP-NH_2/TPPFeCl-NH_2 was coupled to it by reductive amination in DMSO containing an excess of $NaBH_3CN$. The adducts were precipitated by addition of EtOH, then redissolved in water, and purified by Sephadex G-50 gel chromatography (eluted with H_2O) to give Dex-TPP/Dex-TPPFeCl. The content of covalently bound TPP chromophores in dextran polymer could be controlled by varying the reaction conditions and was estimated to be 1–50 μmol/g by measuring the Soret band absorption (410–420 nm). For the experiments described below, Dex-TPP and Dex-TPPFeCl with porphyrin contents of 2.38 and 30.9 μmol/g, respectively,

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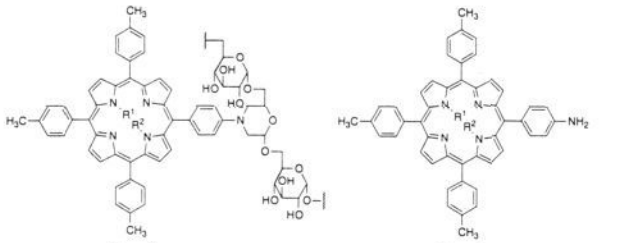

Figure 1. Structures of Dex-TPP, Dex-TPPFeCl, TPP-NH_2, and TPP-FeCl-NH_2.

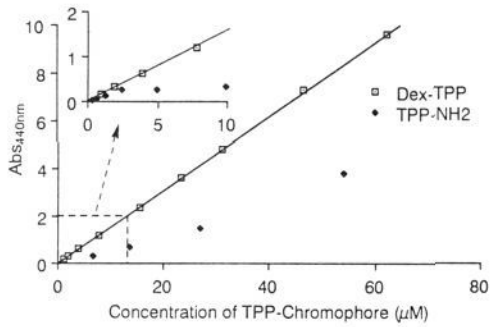

Figure 2. Absorption–concentration relationship of Dex-TPP and TP-P-NH_2 in 10 mM Tris-HCl (pH 7.5) containing 50% DMSO. Absorption at 440 nm was measured in a 1-mm UV cell, and values were multiplied by 10 to prepare the plot. For the inset, absorption was measured in a 10-mm UV cell, and the values were plotted, as obtained.

were used. Both Dex-TPP and Dex-TPPFeCl were quite soluble in water, though both TPP-NH_2 and TPPFeCl-NH_2 are insoluble in water. Solubility in 10 mM Tris-HCl aqueous buffer (pH 7.5) of Dex-TPP and Dex-TPPFeCl was estimated to be 90 mg/mL (corresponding to 214 μM TPP-NH_2 unit) and 34 mg/mL (1050 μM TPPFeCl-NH_2 unit), respectively.

Because ionic water-soluble porphyrins generally stack spontaneously to form dimers and/or higher aggregates in aqueous solutions,[7] Beer's law experiments were carried out at the Soret band (Figure 2). The absorbance of Dex-TPP/Dex-TPPFeCl was found to be directly proportional to concentration over the range investigated, while that of TPP-NH_2/TPPFeCl-NH_2 showed considerable deviation from linearity. These results suggest that each porphyrin/ferric porphyrin chromophore in Dex-TPP/Dex-TPPFeCl exists in a monomeric (naked) state under the conditions, which would be one of the advantages of these compounds over other ionic water-soluble porphyrins.

To examine the function of these porphyrin derivatives, we investigated non-irradiated[5,8] and photoinduced[9] DNA-nicking activity of Dex-TPPFeCl and Dex-TPP, respectively (Figure 3). Dex-TPPFeCl showed DNA-nicking activity in the presence of dithiothreitol,[10] while TPPFeCl-NH_2 surprisingly showed no such activity. This result can be interpreted in terms of formation of the inactive μ-oxo dimer of TPPFeCl-NH_2 under the experimental conditions; Dex-TPPFeCl should not form the μ-oxo dimer because

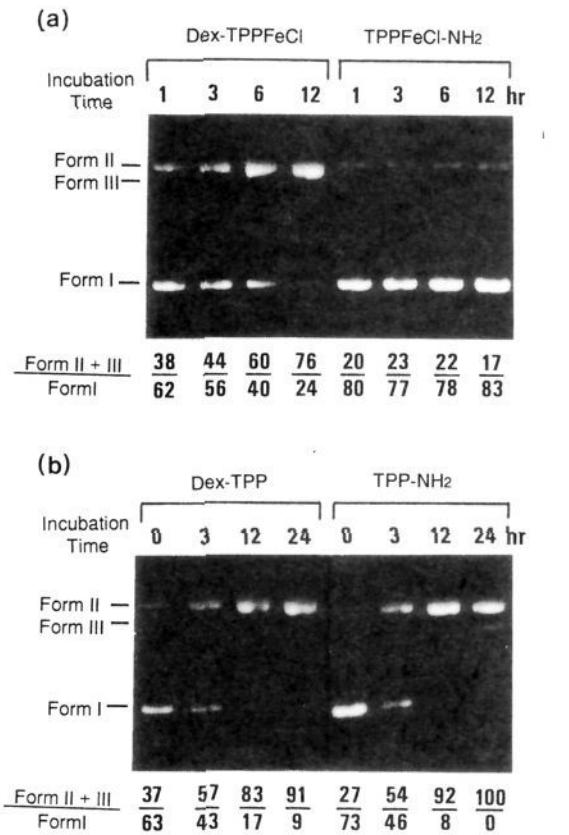

Figure 3. Non-irradiated and photoinduced DNA-nicking activity of Dex-TPPFeCl, TPPFeCl-NH_2, Dex-TPP, and TPP-NH_2. Agarose gel electrophoresis of the reaction mixtures after ethidium bromide staining. (a) Supercoiled form (form I) of pBR322 plasmid (100 μM nucleotides) was incubated with Dex-TPPFeCl or TPPFeCl-NH_2 (10 μM porphyrin chromophore) in 20 mM Tris-HCl (pH 7.7)–0.05 mM EDTA–1 mM dithiothreitol at 37 °C in the dark for the indicated period. For the incubation with TPPFeCl-NH_2, 50% DMF was added. (b) The reaction conditions were the same as described for a, except that the mixture contained 300 μM nucleotide of pBR322 and was incubated in the absence of dithiothreitol at 20 °C under irradiation with a daylight lamp (500 W). The values are the ratio (%) of intact supercoiled DNA form (form I) versus nicked forms [open circular (form II) and linear DNA forms (form III)] quantified by densitometry (whole area spot integration) after ethidium bromide staining. The results were reproducible with experimental errors of less than 15%.

the porphyrin chromophores covalently bound to dextran polymer are located too far apart. Indeed, pH-titration experiments suggested that TPPFeCl-NH_2 forms the μ-oxo dimer (the Soret band shifted pH-dependently: 402 nm at pH 2, 412 nm at pH 7, and 420 nm at pH 12), while Dex-TPPFeCl does not (no shift of the Soret band; 412 nm at pH 2, 7, and 12).[11] In contrast with the case of DNA-nicking activity of Dex-TPPFeCl and TPPFeCl, Dex-TPP and TPP-NH_2 showed comparable activity under the experimental conditions (Figure 3).[10]

Dex-TPP/Dex-TPPFeCl showed a moderate cell-growth-inhibitory effect (ID_{50} value of $\sim$5 μM), though the molecular mechanism of the effect has not been clarified yet.

In conclusion, we have prepared novel non-ionic water-soluble TPP analogs by the use of dextran polymer, i.e., Dex-TPP and Dex-TPPFeCl. These TPP analogs were shown to mimic the function of ionic water-soluble porphyrins in terms of DNA-nicking activity. The advantages of these conjugates over other water-soluble porphyrins include no formation of aggregates or μ-oxo dimer, non-ionic nature (pH-independence), and ease of preparation. The results indicated that the coupling of water-

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(10) The DNA-nicking activity of the compounds was rather weak, probably because of their very low affinity for DNA: no interaction of the compounds with DNA was observed by UV and fluorescence spectral examination or gel electrophoresis.

(11) Another possible explanation is oxidative degradation of TPPFeCl-NH_2; the porphyrin chromophores in Dex-TPPFeCl would only be destroyed by intramolecular processes, not by intermolecular reactions.

insoluble molecules with dextran is effective as a method to confer water solubility. The method should be available for studies of a variety of water-insoluble molecules in aqueous solution.

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Gas-Phase Reactions of Benzenoid Hydrocarbon Ions with Hydrogen Atoms and Molecules: Uncommon Constraints to Reactivity

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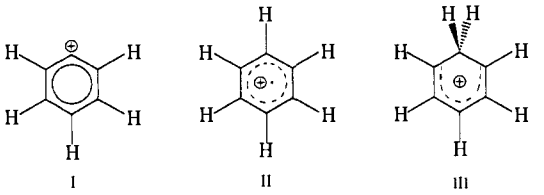
The hydrogen atom, the simplest chemical after the proton, displays in its reactions a selectivity surprising in such a small species. This is evident, for example, in reactions of hydrogen atoms with small hydrocarbon ions which are of fundamental importance in the chemistry of flames, of planetary atmospheres, and of the universe in general where hydrocarbon ions may catalyze the recombination of hydrogen atoms.[1-3] We report here results for the reactions of the benzenoid hydrocarbon ions $C_6H_n^+$ (n = 5-7) with atomic and molecular hydrogen. Remarkably, only two of this series of six reactions, all of which have exothermic channels, were observed to proceed with measurable rates, $C_6H_6^{\bullet+}$ + H$^\bullet$ and $C_6H_5^+$ + H_2, both giving the adduct $C_6H_7^+$. Such constraints to reactivity are most intriguing, particularly from the mechanistic and energetic point of view. Here we shall show that they are consistent with the structures and energies of the benzenoid ions and their possible collision complexes.

Reactions of $C_6H_n^+$ with H$^\bullet$ and H_2 were studied using the SIFT (selected ion flow tube) technique[4,5] at 297 ± 3 K and 0.35 ± 0.01 Torr of helium, unless specified otherwise. Atomic hydrogen was produced in a microwave discharge of H_2 (2-4%) in He.[6,7] $C_6H_5^+$ was generated by 50 eV electron impact upon chlorobenzene or benzene. $C_6H_6^{\bullet+}$ was generated by electron impact upon benzene. $C_6H_7^+$ was formed by proton transfer from CO_2H^+ to benzene introduced into the flow tube and was also formed in the reaction of $C_6H_6^{\bullet+}$ with H$^\bullet$. For these ions, the cyclic structures I-III related to benzene are the lowest energy isomers known[8] and should thus account for most of the $C_6H_n^+$ ion signal in question. For $C_6H_5^+$, two components of differing reactivity were present in accordance with previous studies;[9-11] there was no evidence for the presence of higher energy forms of $C_6H_6^{\bullet+}$ and $C_6H_7^+$.

Table I. Reactions of $C_6H_n^+$ (n = 5-7) with H and H_2

reactants	products	k_{obsd}[a]	k_L[b]	$-\Delta H°_{trf}$[c]	$-\Delta H°_{assn}$[d]
c-$C_6H_5^+$ + H		<0.01	1.9	9	88
l-$C_6H_5^+$ + H		<0.005	1.9	3[e]	42[e]
$C_6H_6^+$ + H	$C_6H_7^+$	0.25[f]	1.9	16	81
$C_6H_7^+$ + H		<0.01	1.9	23	41[g]
c-$C_6H_5^+$ + H_2	$C_6H_7^+$	0.05	1.5		65
l-$C_6H_5^+$ + H_2		<0.001	1.5		24[h]
$C_6H_6^{\bullet+}$ + H_2		<0.001	1.5		18[g]
$C_6H_7^+$ + H_2		<0.001	1.5		13[i]

[a] Observed rate coefficient in units of 10^{-9} cm^3 molecule^{-1} s^{-1}. The accuracy of the rate coefficients is estimated to be ±50% for reactions with H-atoms and ±30% for reactions with H_2. [b] Calculated Langevin collision rate coefficient, in units of 10^{-9} cm^3 molecule^{-1} s^{-1}. [c] Exothermicity of H-atom transfer, $C_6H_x^+ + H \rightarrow C_6H_{x-1}^+ + H_2$, in kcal mol^{-1}. [d] Exothermicity of association, in kcal mol^{-1}. [e] Calculated using $\Delta H°_f(CH_2CCHCHCCH^+)$ = 284 kcal mol^{-1}.[7,12] [f] Invariant over the pressure range 0.2-0.6 Torr. [g] For formation of ionized 1,3-cyclohexadiene. [h] For formation of protonated $CH_3C{\equiv}CC{\equiv}CCH_3$. [i] For formation of protonated 1,3-cyclohexadiene.



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The rate coefficients for the observed reactions are detailed in Table I. Our results for the reactions of $C_6H_5^+$ and $C_6H_6^{\bullet+}$ with H_2 agree with those of Giles et al.,[9] although we favor the assignment of the phenylium ion as the reactive isomer in the reaction of $C_6H_5^+$ with H_2, rather than one of the higher energy linear isomers.[11]

Association is the only exothermic channel for the reactions with H_2. The observed association of c-$C_6H_5^+$ and H_2 can be rationalized as an insertion process involving the H-H bond and the vacant sp^2 orbital on the ipso carbon of the phenylium ion. This association reaction has also been observed at low pressures in FT-ICR experiments, with a rate coefficient $k = 1.5 \times 10^{-11}$ cm^3 molecule^{-1} s^{-1},[11] and thus appears to contain measurable bimolecular and termolecular components, although we have not attempted to verify this experimentally. The absence of association with H_2 for the other ions studied here can be understood since these ions do not have any entirely vacant orbitals or such a localized positive charge.

The reactions with H$^\bullet$ are still more intriguing. Association is more strongly exothermic for c-$C_6H_5^+$ than for $C_6H_6^{\bullet+}$, and yet is seen only in the latter case. Why should c-$C_6H_5^+$, which adds efficiently to H_2 and to many other neutrals,[11] not add detectably to H$^\bullet$? $C_6H_6^{\bullet+}$ possesses more vibrational/rotational degrees of freedom than c-$C_6H_5^+$, but this factor[13] seems insufficient to account for the observed trend in efficiency of association. Furthermore, hydrogen atom transfer reaction 1 is exothermic for all four of the reactant ions included in our study, and yet is not observed for any of these ions.

$$C_6H_n^+ + H^\bullet \rightarrow C_6H_{n-1}^{\bullet+} + H_2 \quad (1)$$

The absence of H-atom transfer from $C_6H_5^+$ to H$^\bullet$ is consistent with the likely collision complex (IV) in which the reacting hydrogen atom never gets sufficiently close to any of the C-H bonds in order to break them and form H_2. The inefficiency of association in this system may arise because the initial collision complex involves an interaction of the hydrogen atom's electron with the vacant σ-orbital upon I, leading to an excited electronic state of the benzene cation. Formation of the ground state requires re-

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