spectrum 2B is a superposition of the spectra of ferri-HRP and ferro-HRP. Thus, irradiation of frozen compound I with 457.9-nm light results in photoreduction first to compound II and ultimately to a mixture of ferri-HRP and ferro-HRP. However, the reaction to produce the two latter species occurs much more rapidly when compouned I is the starting species compared to compound II.

Our finding that B-band excitation of frozen compound I causes extensive photoreduction necessitates a reinterpretation of previous RR experiments. Teraoka and associates[14] have reported RR spectra of frozen compound I obtained with excitation even closer to the B band (441.6 nm). These authors observed two ν_4 bands in the frozen samples at 1381 and 1359 cm^{-1} and a single band at 1377 cm^{-1} after warming the sample to room temperature. The band at 1359 cm^{-1} was attributed to compound I. However, on the basis of the data presented here, the ν_4 bands observed at 1381 and 1359 cm^{-1} in the frozen sample were due to compound II and ferro-HRP, respectively, while that at 1377 cm^{-1} in the warmed sample was due to ferri-HRP. The ν_2 band attributed to compound I at 1590 cm^{-1} arises from compound II and the ν_{10} band at 1636 cm^{-1} is the superposition of that of compound II at 1640 cm^{-1} and that of the peripheral vinyl $\nu(C=C)$ band of ferro-HRP at 1629 cm^{-1}. The iron–His stretching band reported for compound I at 248 cm^{-1} arises instead from that of ferro-HRP. Thus, all of the spectral features attributed to compound I can be accounted for by the photoreduction products described here. Therefore, cryogenic stabilization of compound I is not sufficient to overcome its photolability[15] and RR arguments to support its structure must wait until a method is found of obtaining its spectrum unequivocally. Clark and associates have observed a similar photoreduction of ferrylmyoglobin to ferromyoglobin[18] and recently Kitagawa and associates have reported that HRP compound II is photolabile in solution when excited at 406.7 nm.[19] Thus, photolability is apparently a general property of oxidized forms of heme proteins.

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Observation of the Ultraviolet Absorption Spectrum of Phenyl Radical in the Gas Phase

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Although the phenyl radical is the simplest aromatic radical, its UV absorption spectrum has not yet been established. According to the pioneering works with flash photolysis by Porter and Ward, phenyl radicals have weak absorption bands around 430–530 nm, which are assigned to the n ← π transitions.[1] No other absorption band was found between 290 and 700 nm by them. From ESR studies, the phenyl radical is known to be an n-radical but not a π-radical[2,3] and has almost the same π-electronic system as the parent molecule. It is, therefore, expected that some absorption bands corresponding to the B_{2u} and/or B_{1u} states of benzene exist in the UV region around 250 nm. By pulse-radiolysis technique weak absorptions were found around 255–260 nm in solution and were suggested to be those of phenyl radicals,[4,5] but those spectra were obtained in an indirect way:
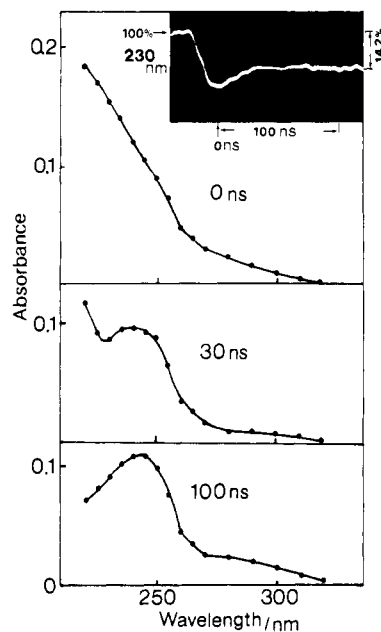

Figure 1. Time-resolved absorption spectra of phenyl radical produced by photolysis of 2.0 torr of chlorobenzene at 193 nm in the presence of 800 torr of nitrogen. A typical oscillogram of the transient absorption at 230 nm is inserted.
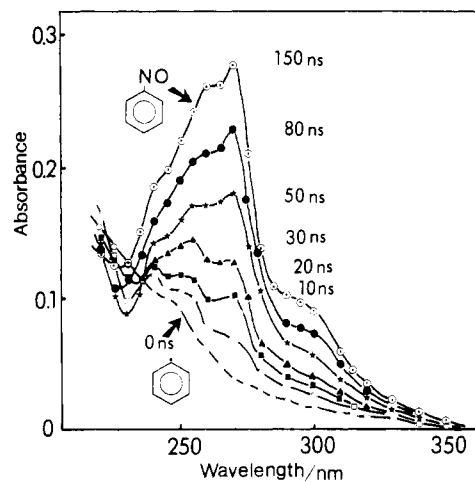

Figure 2. Time-resolved absorption spectra of 3 torr of chlorobenzene in the presence of 20 torr of nitrogen monoxide and 800 torr of nitrogen.

a difference spectrum of individual measurements in the presence and absence of radical scavenger, N_2O, in halogenated benzene solutions.

In this paper, we report for the first time the UV absorption spectrum of phenyl radical in the gas phase directly obtained by nanosecond laser photolysis.[6] Figure 1 shows time-resolved absorption spectra upon excitation of chlorobenzene to the S_3 state at 193 nm. In the photochemistry of chlorobenzene, the quantum yield of phenyl radical has been reported to be close to unity at 184.9 nm with a decomposition rate more than 10^{13} s^{-1}.[9] The formation of phenyl radical is expected to be completed within the exciting pulse width (12 ns). Therefore, the absorption spectrum immediately after excitation must be due to phenyl radical.

In the presence of nitrogen monoxide as a scavenger, a characteristic absorption of nitrosobenzene appears by the reaction

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$C_6H_5 + NO \rightarrow C_6H_5NO$ as shown in Figure 2. This clearly demonstrates that the species formed by 193-nm excitation is phenyl radical.

The broad absorption spectra at 0 ns in Figures 1 and 2 are reasonably assigned to vibrationally highly excited, "hot" phenyl radicals. Its monotonous absorption can be interpreted primarily as a tail of the allowed transition corresponding to the $S_3^{**}(E_{1u})$ ← S_0^{**} of hot benzene. The absorptions of such hot molecules have already been observed in benzene and its derivatives: benzene,[8] toluene,[10,11] and hexafluorobenzene.[12] The available energy E_{avl} of the hot phenyl radical formed is calculated to be 221 kJ/mol.[13] A recent molecular beam photodissociation study has shown that chlorobenzene dissociates at 193 nm to produce vibrationally hot phenyl radicals with an average translational energy of 52 kJ/mol.[15] Assuming a negligible contribution of energy redistribution to rotational energy, we can estimate that the average vibrational energy in phenyl radical is about 169 kJ/mol. This energy corresponds to about 1400 K in the vibrational temperature. The hot phenyl radicals are relaxed by collisions with foreign gases, as can be seen from Figure 1. For example, the fast decay in the absorption time profile at 230 nm is explained in terms of collisional cooling.[7,8,10,12] Consequently, an absorption spectrum at 100 ns in Figure 1 which has a band maximum around 245 nm is assigned to the relaxed phenyl radical.

The relaxed absorption spectrum is in good agreement with the results by pulse-radiolysis studies in solution.[4,5] The spectrum is distinctly different from that of cyclohexadienyl radical[16] or π-complex with halogen atom.[17,18] Although the observed spectra in Figure 1 is close to the spectrum of biphenyl which has a band maximum at 235 nm, a possibility of biphenyl formation within our time scale (≤150 ns) is completely neglected for the following reasons: (1) In the presence of 800 torr of propane, we observed the same transient. Under such conditions, it is known that the final product is benzene, but not biphenyl, in steady-state photochemical studies.[9] (2) On the basis of the known rate constant of $10^{13.0}$ exp(-10 kcal/RT) cm^3 mol^{-1} s^{-1} for the reaction $C_6H_5Br + C_6H_5 \rightarrow C_6H_5C_6H_5 + Br$,[19] biphenyl formation at 100 ns in the present system is estimated to be negligibly small. (3) A linear relation between the transient absorbance at 100 ns vs. the excitation intensity was obtained. If phenyl radicals would recombine themselves to form biphenyl, the absorbance should be proportional to the square of the excitation intensity. Multiphoton processes are also disregarded from this result.

Trapping of nitrosobenzene in the presence of nitrogen monoxide is a simple method to detect phenyl radical and to estimate the quantum yield, because nitrosobenzene has the characteristic absorption with a relatively large molar extinction coefficient (12000 M^{-1} cm^{-1} at 270 nm).[20] Formation of phenyl radical was observed with good yields in the photolysis of bromobenzene, iodobenzene, and pentafluorochlorobenzene. It is interesting to note, however, that the yields of phenyl radical are very low in the case of pentafluorobenzene, α,α,α-trifluorotoluene, and also benzene,[8] although the photon energy is high enough to form phenyl-type radicals. The details will be published elsewhere.

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(13) The available energy is calculated by the following equation. E_{avl} = $h\nu$(193 nm) + E_{int}(300 K) − D_0 = 221 kJ/mol, where E_{int} is the internal energy of the parent molecule and D_0 is the dissociation energy of the C–Cl bond of chlorobenzene (406 kJ/mol).[14]

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Nitric Oxide Adduct of the Binuclear Iron Center in Deoxyhemerythrin from *Phascolopsis gouldii*. Analogue of a Putative Intermediate in the Oxygenation Reaction

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Hemerythrin (Hr) is a non-heme protein containing a binuclear iron site which reversibly binds molecular oxygen in several marine invertebrates.[1] A large body of physical and chemical evidence indicates that an appropriate formulation for the oxygenation reaction is

$$[Fe^{II},Fe^{II}](deoxy) + O_2 \rightleftharpoons [Fe^{III},Fe^{III}O_2^{2-}](oxy)$$
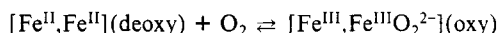
However, X-ray crystallography[2] indicates that dioxygen has direct access to only one of the two iron atoms. Thus, if the formal oxidation state changes shown above are correct, one could postulate the existence of an intermediate between deoxy- and oxyHr, which would be formulated as $[Fe^{II},Fe^{III}O_2^-]$. Such an intermediate has never been detected. However, its transient existence does not seem unreasonable in view of the fact that the two high-spin irons in deoxyHr have usually been described as magnetically uncoupled or nearly so, presumably due to lack of an appropriate bridging ligand. The two high spin irons in oxyHr, on the other hand, are antiferromagnetically coupled through a μ-oxo bridge ($J \sim -75$ cm^{-1}).[3,4]

We report that a nitric oxide adduct of deoxyHr (deoxyNO) from *Phascolopsis gouldii* can be prepared, whose physical and chemical properties are consistent with its formulation as $[Fe^{II},Fe^{III}NO^-]$. Thus, deoxyNO can be viewed as an analogue of the putative intermediate in the oxygenation reaction. The most pertinent feature of the NO adduct is that the irons are magnetically coupled.

Gradual injection over the course of $\sim$5 min of a 1.5–4-fold molar excess of gaseous NO to an anaerobic solution of deoxyHr[5] at room temperature results in development of a pine-green color. The optical spectra are shown in Figure 1. Features in the spectrum of deoxyNO at 408 ($\sim$1000 M^{-1} cm^{-1}), 500 ($\sim$600 M^{-1} cm^{-1}), and 600 nm ($\sim$400 M^{-1} cm^{-1}) are quite different from those of any other derivative of Hr.[1] A sample of deoxyHr (EPR silent) frozen $\sim$9 min after injection of NO elicits an EPR spectrum (Figure 2, top) which is different from those of Hr in oxy and met ($[Fe^{III},Fe^{III}]$, S = 0, EPR silent) or semi-met ($[Fe^{II},Fe^{III}]$, $S = {}^1/_2$, $g_{av} \sim 1.84$) oxidation levels.[1] Double integration of the rather broad, axial signal in Figure 2 vs. a CuSO$_4$ standard yields 0.9 spins/2Fe.[7] The deoxyNO EPR spectrum disappears at $\sim$34

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(7) When this sample was incubated at 4 °C the EPR signal decreased to $\sim$40% of its original intensity after 2 h.