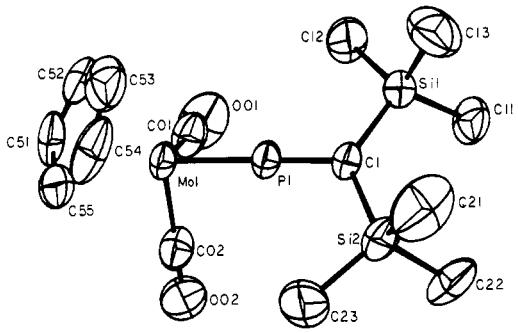
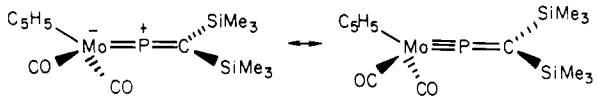


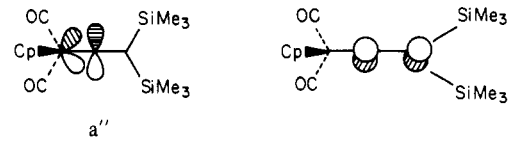
Figure 1. ORTEP view of $[Mo(CO)_2(\eta^1\text{-}P{=}C(SiMe_3)_2(\eta\text{-}C_5H_5)]$ (**1**). Pertinent metric parameters: Mo(1)-C(1) = 2.174 (1), P(1)-C(1) = 1.649 (4) Å; Mo(1)-P(1)-C(1) = 178.3 (2)°, P(1)-C(1)-Si(1) = 116.7 (3)°, P(1)-C(1)-Si(2) = 116.9 (3)°, Si(1)-C(1)-Si(2) = 126.3 (2)°.

bond length of 2.174 (1) Å is significantly shorter than those found in phosphine-Mo complexes (2.40-2.57 Å)[4] and also slightly shorter than the values (2.213 (1) and 2.207 (1) Å) that have been reported for $[Mo(CO)_2(\eta^1\text{-}PR_2)(\eta\text{-}C_5H_5)]$,[5] phosphenium ion complexes in which multiple Mo-P bonding has been implied. The P-C bond length (1.649 (4) Å) is shorter but similar to those in uncoordinated phosphaalkenes which span the range 1.665 (4)-1.72 Å[6] and should therefore be considered a double bond. In addition, the ligand configuration is such that the Mo(1)-P(1)-C(1) angle is almost linear (178.3 (2)°) and the methylene carbon, C(1), is trigonal planar. This plane is essentially orthogonal to the symmetry plane of the $Mo(CO)_2(\eta\text{-}C_5H_5)$ fragment. The electronic structure of **1** can thus be discussed in terms of canonical forms **2** and **3**. The phosphavinylidene structure



2 **3**

2 is isovalent with group 7B vinylidene complexes such as $(C_5H_5)(CO)_2M{=}C{=}CR_2$, M = Mn, Re (**4**). Such a structural assignment gains support from the fact that **1** and **4** are isostructural. As illustrated below, this conformation maximizes the interaction between the HOMO (a″) of the $C_5H_5M(CO)_2$ fragment[7] and a P(3p) orbital. In **3** the Mo-P bonding resembles that in group 6B terminal alkylidyne complexes, $(C_5H_5)(CO)_2M{\equiv}CR$, M = Cr, Mo, W.



a″

The reaction of $(Me_3Si)_2C{=}PCl$ with several organometallate anions is under active investigation as is a study of the reactivity of **1**.

Acknowledgment. We are grateful to the National Science Foundation and the Robert A. Welch Foundation for financial support.

Supplementary Material Available: Tables of bond lengths, bond angles, atomic coordinates, thermal parameters, and structure factors for **1** (35 pages). Ordering information is given on any current masthead page.

UV Resonance Raman Spectroscopy of the Aromatic Amino Acids and Myoglobin

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Resonance Raman spectroscopy in the near-UV and visible spectral region is an important technique for the study of biological molecules.[1] As part of a continuing investigation of the UV resonance Raman (UVRR) spectroscopy of aromatic molecules,[2] we have begun a detailed study of the excitation profiles of phenylalanine, tyrosine, and tryptophan in the region 200-280 nm.[3] The only previously reported UV Raman spectra of tyrosine and tryptophan were excited at 257.3 nm by using a frequency-doubled Ar^+ laser.[4] The shorter excitation wavelengths used in the present work clearly demonstrate the sensitivity and selectivity of UVRR for the study of aromatic amino acids in solution and in proteins. Spectra were easily obtained from solutions of the amino acids and proteins at millimolar concentrations with no interference from fluorescence. Particular excitation wavelengths provide a selective enhancement of the Raman spectrum of one amino acid over another. Photochemical decomposition of the sample was not significant during the individual 30-min Raman spectral measurements.

Typical UVRR spectra of solutions of each of the aromatic amino acids at pH 12 are shown in Figure 1. The spectra were obtained with an instrument based on a Nd/YAG pumped dye laser and associated doubling and mixing crystals, a triple monochromator, and a Reticon detector.[5] The aromatic amino acid samples were recirculated through a jet nozzle during laser irradiation to minimize sample decomposition.[5] The Raman intensities of the samples are referenced to the 932-cm^{-1} symmetric stretch of ClO_4^-, which is used as an internal standard.[6] Comparisons of the UVRR spectra of phenylalanine, tyrosine, and tryptophan to those of toluene, phenol, and indole (not shown) indicate that all of the features in the spectra may be assigned to normal modes of the aromatic rings of the amino acids. As indicated in Figure 1, these spectra were obtained in or near resonance with the $^1L_a \leftarrow {}^1A$ ($\pi^* \leftarrow \pi$) electronic transition. Tryptophan has a higher molar absorptivity than tyrosinate or phenylalanine at 225 nm and is thus a better Raman scatterer at this excitation wavelength.

Different amino acids may be selectively enhanced by changing the excitation wavelength. This is illustrated in Figures 2A and

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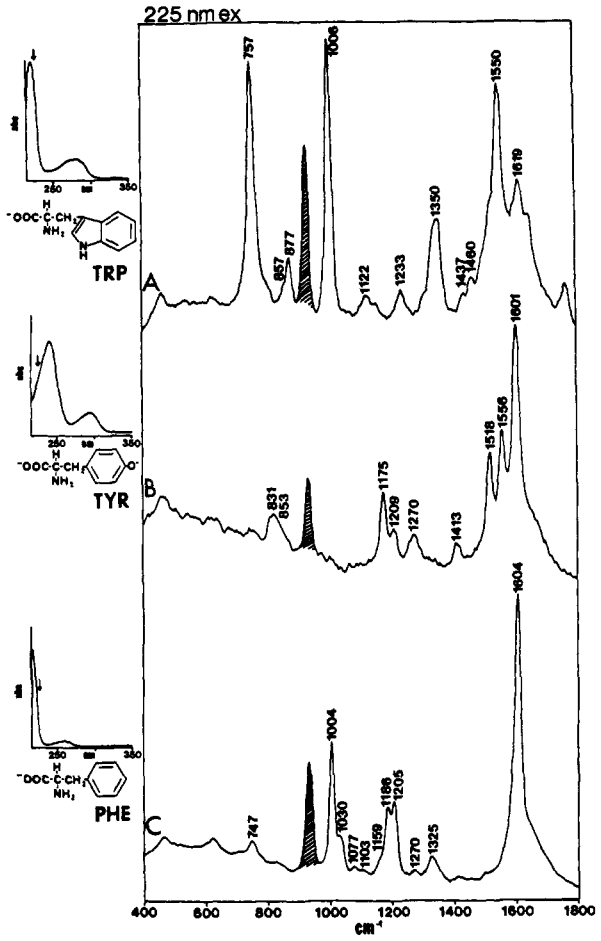

Figure 1. UV resonance Raman spectra of aqueous solutions of the aromatic amino acids at pH 12 with 225.0-nm excitation: (A) 0.001 M tryptophan, 0.5 M $NaClO_4$, (B) 0.005 M tyrosine, 0.2 M $NaClO_4$, (C) 0.005 M phenylalanine, 0.2 M $NaClO_4$. The 932-cm^{-1} band of the ClO_4^- internal standard is indicated by shading. UV electronic absorption spectra and an arrow indicating the excitation wavelength are shown above the structure of each of the amino acids. Each spectrum was accumulated in 30 min with 20 mW of average power at a 20-Hz pulse repetition rate; spectral resolution = 14 cm^{-1}.

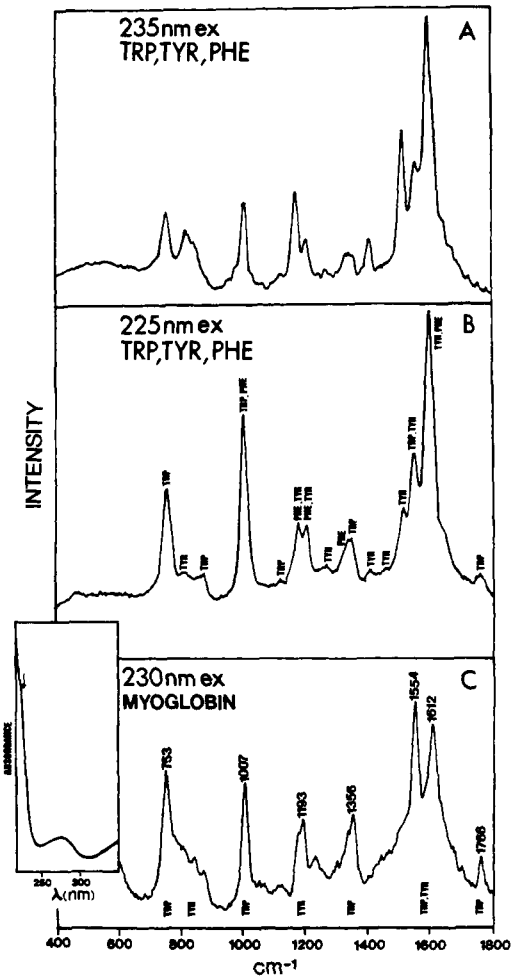

Figure 2. UV resonance Raman spectra of an aromatic amino acid mixture and metmyoglobin: (A) Aromatic amino acid mixture: [Phe] = 0.005 M, [Tyr] = 0.002 M, [Trp] = 0.001 M, pH 12; 235.0-nm excitation. (B) Same as (A) but at 225.0-nm excitation; the instrument parameters are as in Figure 1. (C) UV resonance Raman and absorption spectrum of sperm whale metmyoglobin; 0.2 mM metmyoglobin in 0.01 M pH 6.8 phosphate buffer; Excitation wavelength = 230 nm. The spectrum was obtained in 15 min with 3 mW of average power at a 20-Hz pulse repetition rate; spectral resolution = 14 cm^{-1}.

2B where UVRR spectra of a mixture of all three amino acids are shown. With 225-nm excitation (Figure 2B) the spectrum is dominated by bands assignable to tryptophan (despite the lower tryptophan concentration), except for the strong feature due to phenylalanine and tyrosine at 1600 cm^{-1}. The bands due to each of the amino acids are indicated in the figure. Excitation at 235 nm occurs at the absorption maximum of tyrosinate (pH 12), and a relative enhancement occurs for the tyrosinate Raman peaks of the mixture (Figure 2A).

Normal Raman studies of proteins have indicated that particular Raman bands of the aromatic amino acids are environmentally sensitive.[1b,c,7,8] These environmentally sensitive bands are enhanced with UV excitation. For example, the intensity ratio of the doublet at 850 and 830 cm^{-1} of tyrosine (not resolved at the spectral resolution used for Figure 1B) depends upon pH and solvent properties such as polarity and hydrogen-bonding ability.[7] This doublet has been used to examine the extent of hydrogen bonding of tyrosine residues in several proteins[9] to classify the tyrosines as either buried within the protein or exposed to the aqueous medium.[1b,9] With excitation at 235 nm, we observe $I_{830\ cm^{-1}}/I_{850\ cm^{-1}}$ ratios of 0.87 and 1.2 for protonated (pH 7) and deprotonated (pH 12) tyrosine, respectively, compared to ratios of 0.8 and 1.4 observed in normal visible-wavelength Raman studies.[7] In resonance, however, the ratio also depends upon the excitation wavelength.

The resonance Raman excitation profiles of tyrosine and tryptophan between 217 and 260 nm are highly structured[3] with peaks showing full-widths half-maxima of ca. 3 nm. Because aromatic amino acids in different protein microenvironments typically show absorption spectral shifts of ca. 5 nm,[10] it appears possible to selectively enhance individual tyrosine residues in a protein. Thus, it may be possible to use this ratio as a probe of individual tyrosine environment once the wavelength dependence of the doublet intensity ratio is determined from model compound studies and studies of proteins with known tyrosine environments.

UV excitation selectively enhances Raman peaks of aromatic amino acids in proteins. Figure 2C shows the absorption and UVRR spectrum of a 0.2 mM solution of sperm whale metmyoglobin at pH 6.8 in phosphate buffer excited at 230 nm. The protein sample was gently recirculated through a quartz capillary to avoid denaturation. All of the major features in the spectrum derive from tryptophan and tyrosine residues as indicated in Figure 2. Phenylalanine is only weakly enhanced with 230-nm excitation, while the enhancements of other protein residues and the peptide backbone vibrations are negligible at 230 nm relative to that of

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tyrosine and tryptophan. No heme vibrations are observed even though the heme absorption at 230 nm is comparable to that of the three tyrosines and two tryptophans present in the protein.[11] Indeed, the intense heme resonance Raman intensities which occur with visible-wavelength excitation have prevented normal Raman studies of the globin protein.

It appears possible to selectively excite particular aromatic amino residues within a protein. Our recent pH study[12] of the UVRR spectra of myoglobin was able to detect the deprotonation of the single tyrosine in myoglobin which has a pK = 10.5; we were able to selectively enhance this tyrosinate residue. The other two tyrosines in myoglobin have higher pK values.[11]

These results indicate that UVRR spectroscopy shows promise as a new technique for the study of aromatic amino acid residues in proteins. The aromatic residues may be examined free of interference from Raman bands of other residues; histidine and N-methylacetamide (a model for a peptide bond), for example, are not sufficiently enhanced with 225-nm excitation to be detected at concentrations even 100 times greater than that of the aromatic amino acids.[12]

Acknowledgment. We gratefully acknowledge support for this study from NSF Instrumentation Grant PCM-8115738 and NIH Grant 1 R01 GM 30741-02.

Note Added in Proof. While this paper was in press another UV Raman study of aromatic amino acids appeared.[13] Although shorter excitation wavelengths were used, the conclusions are similar to those reported here.

Registry No. Phe, 63-91-2; Tyr, 60-18-4; Trp, 73-22-3.

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Temperature Dependence of Long-Range Electron Transfer in [Zn,Fe^{III}] Hybrid Hemoglobin

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We have shown recently[1] that mixed-metal, [M,Fe], hybrid hemoglobins[2] can be used to study long-range electron transfer[3] between chromophores that are rigidly held at fixed *and* crystallographically known distance and orientation.[4] In these experiments hemoglobin chains of one type (α or β) are subustituted with closed-shell zinc protoporphyrin (ZnP), and chains of the opposite type are oxidized to the aquoferriheme (Fe^{III}P) state. Electron transfer occurs within the α_1-β_2 electron-transfer complex between chromophores that are separated by two heme pocket walls and are at a metal-metal distance of 25 Å. Flash photoexcitation of ZnP to its triplet state initiates the primary process,[5]

$$^3\text{ZnP} + \text{Fe}^{III}\text{P} \xrightarrow{k_t} (\text{ZnP})^+ + \text{Fe}^{II}\text{P} \quad \Delta E_0' \approx 0.8 \text{ V} \qquad \text{(a)}$$

thereby forming an intermediate that returns to the ground state by back electron transfer from Fe^{II}P to the thermalized cation radical, ZnP$^+$,

$$(\text{ZnP}^+) + \text{Fe}^{II}\text{P} \xrightarrow{k_b} \text{ZnP} + \text{FeP} \quad \Delta E_0' \approx 1.0 \text{ V} \qquad \text{(b)}$$

We now report the temperature dependence of k_t, which indicates that reaction a involves electron and nuclear tunnelling.[3]

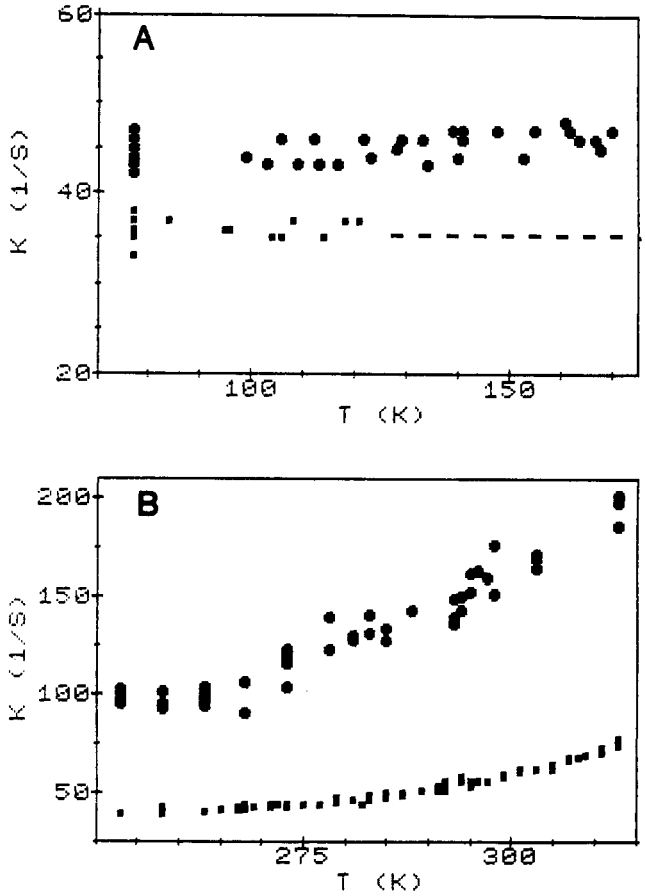

Figure 1. Temperature dependence of the ^{3}ZnP decay rate constants for the oxidized [α(Fe^{III}H$_2$O);β(Zn)] (•) and reduced [α(Fe^{II});β(Zn)] (■) hemoglobin hybrids. (A) Data between 77 and 170 K. The dotted line indicates that the rate constant for the reduced species is invariant in this range, being the same at 250 K as at 120 K. (B) Data between 250 and 315 K. Data for the reduced and oxidized hybrids represent 5 and 16 independently prepared samples, respectively. See ref 6 for details of experimental procedures and conditions.

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(5) (a) The concentration of ^{3}ZnP subsequent to flash excitation was monitored, as described in ref 1. Improvements in technique have led to some differences between room-temperature rates reported here and in ref 1. (b) The redox potentials for processes a and b differ substantially from those in ref 1, to the extent that $\Delta E_0'$ (b) > $\Delta E_0'$ (*a*), not the reverse. The original values employed the reduction potential for (ZnP)$^+$ incorporated into horseradish peroxidase. A more appropriate value is that for (ZnP)$^+$ incorporated into hemoglobin, and to our surprise, work in progress indicates that a much larger value than that for peroxidase, namely $E_0'((\text{ZnP})^+) \approx +1.2$ V (NHE) for e^- + (ZnHb)$^+$ ⇌ ZnHb. In addition we take $\Delta E_T = E(^3\text{ZnP}) - E(\text{ZnP}) = 1.84$ V (ref 5c) and $E_0'(\text{Hb}^+) = 0.15$ V (NHE, ref 5d) in the equations—$\Delta E_0'(\text{a}) = \Delta E_T - E_0'((\text{ZnP})^+) + E_0'(\text{Hb}^+)$; $\Delta E_0'(\text{b}) = \Delta E_t - \Delta E_0'(\text{a})$. (c) Stanford, M. A.; Hoffman, B. M. *J. Am. Soc.* **1981**, *103*, 4104-4114. (d) For example: Bull, C.; Hoffman, B. M. Proc. *Natl. Acad. Sci. U.S.A* **1975**, *72*, 3382-3386.

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