structure was determined by X-ray techniques. Crystal data: space group $P\bar{1}$; a = 10.626 (7), b = 14.553 (6), c = 17.335 (8) Å; α = 109.57, β = 97.19, γ = 95.85 (5)°; V = 2476.0 Å^{-3}; Z = 2; $F(000)$ = 1032; μ (Mo Kα) = 2.0 cm^{-1}. The crystal structure was solved by direct methods (MULTAN 77) and refined by block-diagonal least-squares techniques (coordinates and anisotropic thermal parameters for the nonhydrogen atoms) to a current R factor of 0.10.[14,15]

The ligand is folded around the ammonium group, the N-O distances to the two phosphine oxide groups and the central oxygen atom of the diethylene glycol unit being the shortest. Triphenylphosphine oxide is known to have very high formation constants in hydrogen-bond formation.[16] The trifluoromethanesulfonate anion is remote from the complexed ammonium ion. As observed in the complex of the PF_6 salt of DL-phenylglycine methyl ester and a chiral macrocyclic polyether,[4b] the phenyl group and the ester function of the amino acid ester in **3** were parallel to aromatic groups of the ligand. Thus the hypothesis[4b] of charge transfer and/or hydrophobic interactions contributing to the stability of the complex seems to be valid.

Isolation of the adduct **3** is the first example of a characterized complex of an amino acid ester salt and an acyclic ligand. The ease of preparation of **2** should allow access to new ligands containing chiral carbon or phosphorus centers which may be used to effect enantioselective molecular complexation.

Supplementary Material Available: Atomic coordinates of the bonded atoms and bond distances and angles of the osculate atoms of the asymmetric unit (17 pages). Ordering information is given on any current masthead page.

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(8) Generated by reaction of **1b** with 1 equiv of sodium ethoxide in ethanol.
(9) Initially obtained as the clathrate with CH_2Cl_2, mp 123–124 °C. The solvent molecule is lost after heating at 130 °C in vacuo for 3 h.
(10) Correct elemental analyses and spectral data were obtained for all compounds.
(11) Quantitative results of solvent extraction experiments with **2** and transition metal ions will be reported elsewhere.
(12) Extraction of NH_4^+ in this manner resulted in a protonated species derived from **2** (NMR). Attempts at isolation as the ClO_4^- or trifluoromethanesulfonate salt gave an amorphous solid which could not be satisfactorily characterized by elemental analysis, but did not contain nitrogen.
(13) Prepared from DL-phenylglycine ethyl ester hydrochloride and silver trifluoromethanesulfonate in 88% yield, mp 155–156 °C.
(14) Key structural parameters follow. Distances: N-O(1), 2.69 (2); N-O(2), 2.65 (2); N-O(4), 2.82 (2); N-O(5), 3.24 (3); N-O(3), 3.25 (2); N-C(6), 1.39 (2) Å. Angles: O(1)-N-O(2), 97; O(1)-N-O(4), 109; O(2)-N-O(4), 115; O(1)-N-C(6), 112; O(2)-N-C(6), 104; O(4)-N-C(6), 118°.
(15) Owing to the high thermal motion of the molecule, it is not possible to locate the hydrogen atoms unambiguously. A low temperature X-ray study is underway and will be reported in a full paper.
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(17) We tentatively account for the selectivity with regard to complexation of isopropylamine in terms of hydrophobic interactions of the isopropyl group with phenyl groups of the ligand.

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Resonance Raman Evidence for Charge-Transfer Interactions of Phenols with the Flavin Mononucleotide of Old Yellow Enzyme

Sir:

During the reaction of various flavoenzymes with substrates, nonsemiquinone-type intermediates often appear and show a broad absorption band at longer wavelengths. These reaction intermediates are usually assigned to charge-transfer complexes of oxidized or reduced enzymes with substrate or product, but the details are not clear. In the case of Old Yellow Enzyme (NADPH oxidoreductase, EC 9.6.99.1) (OYE) which has a flavin mononucleotide (FMN) per monomer, many phenolic compounds are bound tightly to OYE when the flavin is in the oxidized state, giving rise to a long wavelength absorption in the same manner as the reaction intermediates,[1] and a systematic correlation exists between λ_{max} of the complexes and σ_{para} of Hammet constant of the para substituents of phenol.[2] Existence of such correlation suggests that the phenol is the charge-transfer donor and oxidized flavin of the enzyme is the acceptor. Although phenols are inhibitors of the enzymatic reaction, physicochemical investigation of the complexes is substantially necessary to determine chemical properties of the coenzymes and also to elucidate the catalytic mechanism of the enzymatic reaction.

It was recently demonstrated that the resonance CARS[3,4] and spontaneous resonance Raman scattering of flavoproteins[5] selectively revealed the vibrational frequencies of in-plane modes of isoalloxazine without interference by vibrations of the apoenzyme. The Raman lines were assigned empirically based on the data of isotopic frequency shift for the ^{13}C- and ^{15}N-labeled riboflavin.[6] Since resonance Raman spectroscopy offers, in principle, the vibrational frequencies of chromophoric group, excitation of Raman scattering of the OYE-Phenol complex at the long wavelength band might provide Raman lines associated with its chromophore, i.e., internal modes of both flavin and phenol besides the flavin-phenol stretching mode, if the band were caused by a charge-transfer transition. Thus it was expected that the resonance Raman spectroscopy could reveal details of the flavin-ligand interactions, although observation of their spectra was generally very difficult because of strong fluorescence. In the present study we successfully applied the technique to the OYE-pentafluorophenol complex by exciting Raman scattering in the long wavelength band of the complex, observing the Raman lines characteristic of the charge-transfer complex.

OYE from beer yeast (M_r = 4.9 × 10^4) and its apoprotein were purified according to Abramovitz and Massey.[2,7] Enzyme concentration was determined spectrophotometrically using ϵ_M = 1.06 × 10^4 M^{-1} cm^{-1} at 462 nm[7] and the concentration of apoprotein was determined by fluorometric titration of FMN. Excess apoprotein was added to the enzyme solution to remove fluorescence. Pentafluorophenol (F_5Ph) was purchased from Wako Pure Chemicals. Raman scattering was excited by an Ar-Kr mixed-gas laser (Spectra Physics, Model 162) and recorded with a JEOL-400D Raman spectrometer equipped with a cooled HTV-R649 photomultiplier. For Raman experiments, ~300 μL of sample solution was put in a longitudinal type cell placed in a thermostated cell holder and was kept at 20 ± 2 °C during the measurements. The laser power at sample point was ~35 mW. Before and after the Raman experiments an identical value of specific activity of OYE was obtained and the absorption spectrum was unaltered.

Figure 1 shows the Raman spectra of OYE, F_5Ph, and their complex excited at 568.2 nm. The concentration of OYE or F_5Ph was the same as in their mixed solution and all of the samples commonly contained 3.3% (w/w) of $(NH_4)_2SO_4$.

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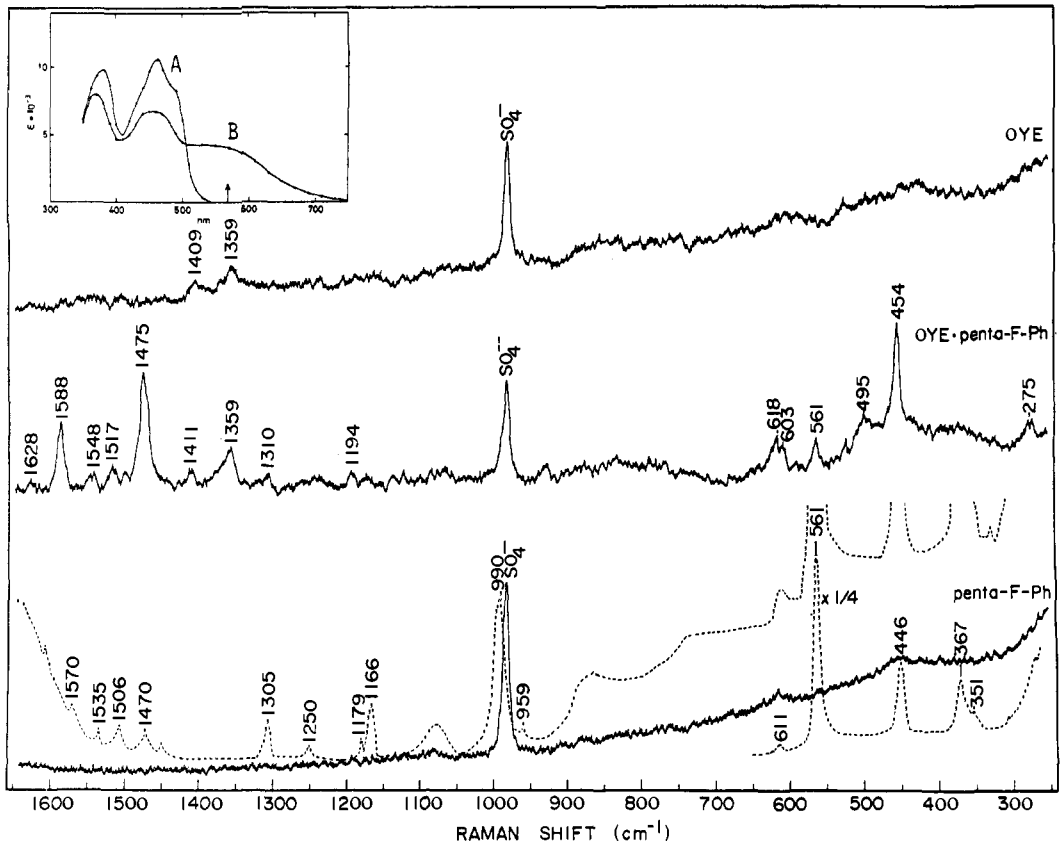

Figure 1. The resonance Raman spectra of OYE (top), OYE-F_5Ph complex (OYE·penta-F-Ph, middle), and F_5Ph (penta-F-Ph, bottom) excited at 568.2 nm (35 mW). All of the samples commonly contained 3.3% $(NH_4)_2SO_4$ and the solvent was 0.1 M sodium phosphate buffer at pH 7.0. The concentrations of OYE and F_5Ph were 8.77×10^{-4} M and 1.84×10^{-3} M, respectively, in both their pure solutions and mixed solution. The concentration of excess apoprotein was 6.15×10^{-5} M for both OYE and OYE-F_5Ph solutions. The Raman line designated as SO_4^{--} indicates the Raman line of SO_4^{2-} at 981 cm^{-1}. The broken line at the bottom was observed for the hundredfold concentrated solution (0.112 mM) of F_5Ph in 0.1 M sodium phosphate buffer without $(NH_4)_2SO_4$. The inset figure displays the absorption spectra of OYE (A) and OYE-F_5Ph (B) in 0.1 M sodium phosphate buffer at pH 7.0. Concentrations of OYE and F_5Ph were 2.3×10^{-5} M and 2.5×10^{-4} M, respectively. An arrow indicates the excitation wavelength of Raman scattering.

Therefore the 981-cm^{-1} line of SO_4^{2-} could serve as an internal standard for the intensity of Raman lines. The resonance Raman spectra of OYE and its complex were identical in the presence and absence of $(NH_4)_2SO_4$. All of the Raman lines were not shifted in D_2O compared with those in H_2O. The Raman spectrum of the hundredfold concentrated F_5Ph solution is also exhibited in Figure 1 with a broken line. The inset of Figure 1 gives the absorption spectra of free OYE and its complex with F_5Ph.

Free OYE had no absorption around 550-700 nm and accordingly only two weak Raman lines of isoalloxazine were observed at 1409 and 1359 cm^{-1} upon excitation at 568.2 nm. On the other hand, the complex gave a broad absorption band around 550-750 nm characteristic of the charge-transfer complex of OYE as Abramovitz and Massey found,[2] and its Raman spectrum excited at 568.2 nm in fact included the Raman lines of both FMN and the interacting phenol; the lines at 1475, 1310, 561, and 454 cm^{-1} were assigned to F_5Ph since these lines were seen for free F_5Ph but not for other flavoproteins.[3-6] Furthermore, these lines were absent in the charge-transfer complexes of other phenol derivatives with OYE. All other lines observed were reasonably attributed to vibrations of isoalloxazine. It was noted that the relative intensity of Raman lines was significantly different for the complex and free OYE or F_5Ph. Particularly, the Raman lines of F_5Ph at 1475 and 454 cm^{-1} were markedly enhanced, whereas that at 561 cm^{-1} was less intensified. As for the flavin moiety, the intensity enhancement of the 1588-cm^{-1} line was conspicuous.

This selective intensity enhancement of Raman lines of isoalloxazine and F_5Ph upon excitation at the charge-transfer band may imply a particular mode of their interaction. The 1588-cm^{-1} line of flavin is known to involve the vibrational displacement of the C(4a) and N(5) atoms of isoalloxazine.[6] The invariance of Raman frequencies upon N(3) deuteration in D_2O indicates that the vibrations involving the C(2)-N(3)H-C(4) linkage were not involved in the present spectrum and therefore were not resonance enhanced by the charge-transfer interaction. These suggest that the charge-transfer interaction occurs in the C(4a)-N(5) region but does not involve the N(3)-H bond. This is consistent with the results of quantum-mechanical calculation by Song et al.[8] which pointed out that the frontier orbital density of flavin is notably higher at N(5) than at other positions and that, therefore, N(5) is most likely to be attacked by electron donors. The frontier orbital density at C(4a) is calculated to be the position of next intensity, again consistent with the present results.

The 561- and 1470-cm^{-1} lines of F_5Ph are assigned to the totally symmetric C-F stretching and the benzene breathing mode, respectively,[9,10] although there is considerable mode-mixing. The latter frequency of the complex is appreciably shifted from that of free F_5Ph (see Figure 1). The prominent peak of the complex at 454 cm^{-1} is presumably associated with the ν_{6a}-like ring-deformation mode. All of the observed phenol modes belong to the A_1 symmetry of the C_{2v} group, i.e. the in-plane modes symmetric to the C_2 axis.

It was noticed that the present set of observed Raman lines of the phenolate anion completely differs from the set of the Raman lines which were observed for a few Fe^{3+}-phenolate complexes upon excitation at their charge-transfer transition;

for hemerythrin[11] and protocatechuate-3,4-dioxygenase,[12] the Raman lines of phenolate anion were observed at ~1600, 1500, 1260, and 1170 cm^{-1}. The CO stretching mode of phenolate anion at 1260 cm^{-1} was intense in those complexes whereas it was missing for the present complex. This implies a substantial difference in the interaction mode of phenolate anion for the two types of complexes. An interaction in the parallel plane arrangement may be suggested for the present case. Detailed analysis of the binding mode of the phenolate anion to the flavin coenzyme of OYE as well as the resonance Raman spectra of the OYE complexes of other substituted phenols and their excitation profiles will be reported separately.

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Electron Transfer Processes. 21. The Use of α-Halo Nitroalkanes as Ketone Equivalents in Condensation Reactions

Sir:

The reaction of a variety of enolate-type anions with 2-chloro-2-nitropropane has been demonstrated to proceed by a free radical chain mechanism[1-4] which has been termed $S_{RN}1$ (Scheme I).[5] Only a few examples of secondary enolate anions have been successfully employed. One example is diethyl malonate, which in sodium ethoxide/ethanol gives a 54% yield of 1-nitro-1-methylethyl ethyl malonic ester or a 44% yield of $(CH_3)_2C{=}C(CO_2C_2H_5)$.[6] Other primary or secondary enolate anions, such as cycloalkanone enolate anions, give little or no coupling with 2-chloro-2-nitropropane in sodium ethoxide/ethanol or potassium *tert*-butoxide in *tert*-butyl alcohol, DMF, or Me_2SO solutions even when irradiated. We herein report conditions which allow a wide variety of primary and secondary enolate anions to be condensed with 2-chloro-2-nitropropane by the $S_{RN}1$ process to yield the products of a controlled crossed aldol condensation.

Table I. Reaction of Cyclohexanone Enolate Anion with 2-Chloro-2-nitropropane (Li^+, THF)

mol ratio[a]	temp (°C) and time	reaction products (%) Cl⁻	1	2	3	cyclohexylidenyl-cyclohexanone
1:1:1	25°, 15 m	56	9	18	4	trace
1:1:1	20°, 1 h	55	26[b]	20[b]	3	0
1:1:1	45°, 30 m	75	6	16	17	trace
1:1:2	45°, 30 m	96	0	28	~10	18
2:1:2	45°, 30 m	100	6	38	12	12
1:1:1	45°, 1 h	61	5	27	13	trace
1:1:1[c]	45°, 2 h	20	0	0	0	15

[a] Ratio of cyclohexanone:2-chloro-2-nitropropane:lithium diisopropylamide; reactions were generally conducted on a 0.05 mol scale. [b] 46% yield of **1** isolated by distillation of aqueous (basic) hydrosylate. [c] Containing 5.5 mol % of di-*tert*-butyl nitroxide.

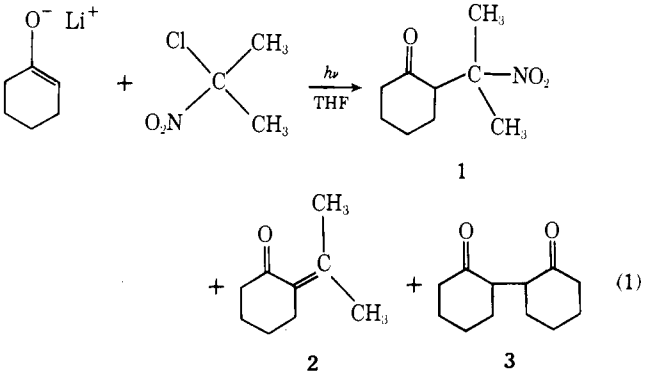
Scheme I. $S_{RN}1$ Mechanism (E^- = Enolate Anion)

$$(CH_3)_2C(Cl)NO_2^{-}\cdot \rightarrow (CH_3)_2\dot{C}NO_2 + Cl^-$$

$$(CH_3)_2\dot{C}NO_2 + E^- \rightarrow (CH_3)_2C(NO_2)E^{-}\cdot$$

$$(CH_3)_2C(NO_2)E^{-}\cdot + (CH_3)_2C(Cl)NO_2 = (CH_3)_2C(Cl)NO_2^{-}\cdot + (CH_3)_2C(NO_2)E$$

Treatment of cyclohexanone with lithium diisopropylamide in THF followed by the addition of 2-chloro-2-nitropropane gives in 1 h at 10–45 °C a reasonable conversion to **1**, **2**, and **3** with only traces of cyclohexylidenylcyclohexanone under the proper conditions (Table I). Yields of **1** plus **2** in the range of 50% are easily achieved. Since the isopropylidene ketone (**2**) is formed from **1** by E2 elimination, the ratio of **1** to **2** depends upon reaction and isolation conditions. Under the best conditions we have observed a ratio of β-nitro ketone/isopropylidene ketone ~5:1 from several ketones. The use of an excess of base in reaction 1 converts **1** to **2** quantitatively, but appreciable amounts of cyclohexylidenylcyclohexanone are now formed. If the reaction product is simply treated with an excess of water and resulting basic solution of water and organic material codistilled, **1** undergoes elimination to yield **2** which is readily isolated in pure form by a redistillation of the organic distillate. Neutralization of the product from reaction 1 followed by solvent extraction and distillation gives **2**, **1** (bp 98 °C (0.3 Torr), mp 69–70 °C), and **3** as a higher boiling fraction.



(1)

The conversion of an enolate anion into the α-alkylidene ketone, the β-nitro ketone, and the enolate dimer appears to be a general reaction. All of these materials are of interest for further synthetic transformations and are often not readily available. Since a wide variety of α-halo nitroalkanes are readily available and, in fact, can be made from the appropriate ketone,[7-12] this $S_{RN}1$ reaction of primary and secondary en-

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