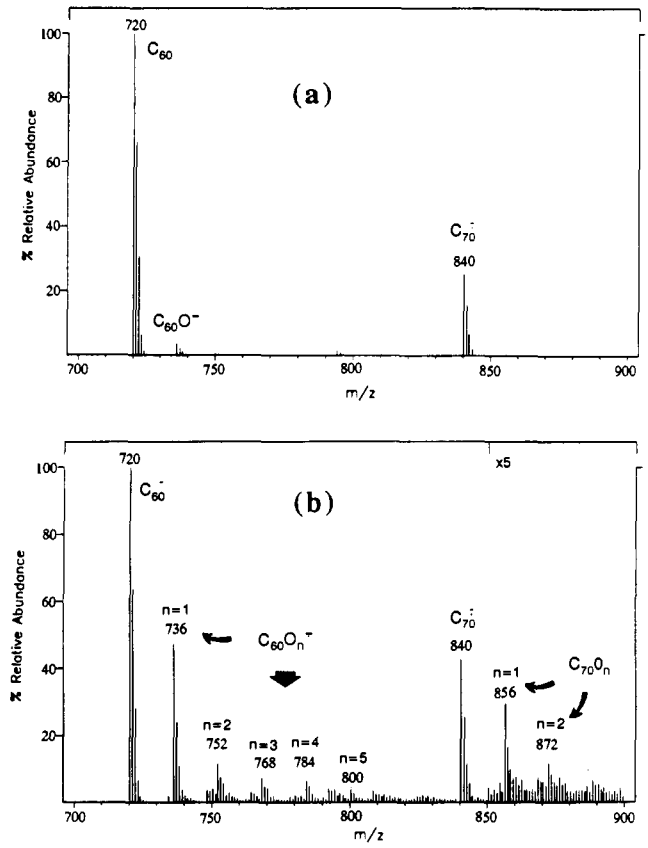

Figure 3. (a) Electron-attachment mass spectrum of ether washings of raw soot extract; (b) after 45-min irradiation, showing the $(C_{60}O_n)^-$ and $(C_{70}O_n)^-$ adducts.

adduct. These results clearly indicate that the oxides are created outside the mass spectrometer.

Even more significant is the observation that the M + 16 adduct is the first member of a series of derivatives we interpret as sequential cyclopropanation and epoxidation products of monoxides. For example, Figure 2a shows the electron-attachment spectrum of benzene extracts of graphitic soot. Ultraviolet irradiation for 1 h (Figure 2b) not only increased the $(M + O)^-$ peaks substantially (with appropriate isotopic peaks at higher mass) but also produced peaks that correspond to $(C_{60}O + (CH_2)_n)^-$ where n = 1, 2, 3, 4, 5, and 6 and $(C_{70}O + (CH_2)_n)^-$ where n = 1 and 2. However, these products were not increased upon irradiation of fullerene mixtures that were thoroughly washed with ether. This suggests that the ether washing removed an unidentified compound that participated in the photochemical reaction of the fullerenes.

In an attempt to concentrate the reactive compound we irradiated ether washings that contained small amounts of C_{60} and C_{70} and discovered a new photochemical reaction. As expected, we saw increased yields of $C_{60}O$ and $C_{70}O$. However, rather than sequential CH_2 additions we observed sequential oxygen atom additions; the monoxides were accompanied by peaks corresponding to $C_{60}O_n$, where n = 2, 3, 4, and 5, and $C_{70}O_n$, where n = 2. Mass spectra of these ether washings recorded before and after irradiation are shown in Figure 3. Similar spectra were obtained on irradiation of ether washings redissolved in benzene, thus discounting ether as the oxygen source.

Low-mass hydrogen containing compounds, relative to C_{60}, are produced in the arc-welding synthesis, and their effect on the aforementioned photochemistry is being investigated. Isolation and characterization of the adducts themselves are also underway.

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The Electron Input to Cytochrome *c* Oxidase from Cytochrome *c*[†,‡]

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The intermolecular electron transfer (ET) between cytochrome *c* and cytochrome *c* oxidase (CcO) has attracted considerable attention in recent years.[1-6] It is still controversial, however, which one of the two low-potential centers (Cu_A and cytochrome *a*) is the primary electron acceptor in the native form of the enzyme. The determination of the initial electron acceptor is of particular interest because of the possible involvement of one of these two centers in proton pumping.[7,8]

In this communication, we report kinetic studies of the intracomplex ET between cytochrome *c* and CcO in both the native and Cu_A-depleted forms using the laser flash photolysis technique recently developed by Hazzard et al.[5] In this experiment, cytochrome *c* is rapidly reduced by flavin semiquinone generated by the laser excitation of 5-deazariboflavin (5-DRF) in the presence of EDTA and the ET from ferrocytochrome *c* to CcO is followed by optical spectroscopy.

Figure 1 shows the kinetic data observed for the intracomplex ET between bovine cytochrome *c* and fully oxidized native bovine CcO at 1:1 molar ratio and 110 mM ionic strength.[9] The reduction of ferricytochrome *c* by 5-DRF semiquinone and its subsequent reoxidation by CcO were monitored at 550 nm (Figure 1A). The kinetic trace is biphasic and fits well to a sum of two exponentials. A rate constant of 1250 ± 63 s^{-1} is obtained for the fast phase with an amplitude corresponding to 75% of the total signal change. The reduction of cytochrome *a* was followed at 604 nm (Figure 1B). This reduction is also biphasic with a rate constant of 1300 ± 45 s^{-1} for the fast phase. Thus, there is excellent correspondence between the reoxidation of the ferrocytochrome *c* and the reduction of cytochrome *a* in the fast phase.

Under otherwise identical conditions, the Cu_A-depleted CcO[9] also displays biphasic kinetics for the reoxidation of ferrocytochrome *c* and reduction of cytochrome *a* (Figure 2). Whereas the rate constants for the slow phase are the same for both the native and the Cu_A-depleted enzymes (slower than 80 s^{-1}), the rate constant for the fast phase for the Cu_A-depleted protein is approximately 25% that of the native enzyme. The fitting gives 300 ± 20 s^{-1} (at 604 nm) and 320 ± 18 s^{-1} (at 550 nm).

The rate constant for the fast kinetic phase (k_{obsd}) is dependent on the concentration of CcO.[5] This dependence is hyperbolic for

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[‡] Abbreviations: ET, electron transfer; CcO, cytochrome *c* oxidase; 5-DRF, 5-deazariboflavin.

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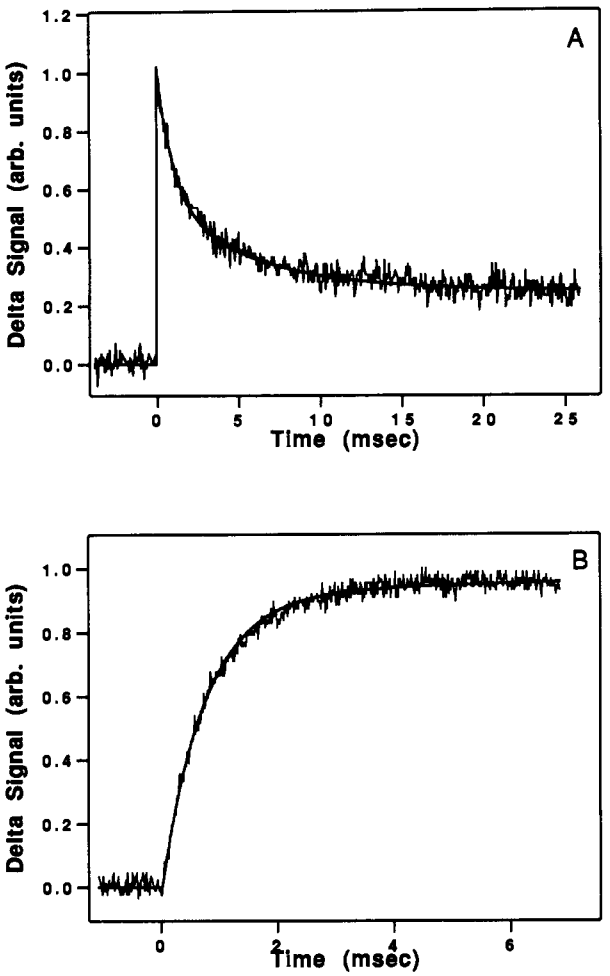

Figure 1. Intracomplex ET between cytochrome *c* reduced by photogenerated flavin semiquinone and native CcO. Native bovine CcO (20 μM) and 20 μM bovine cytochrome *c* were added in 5 mM Tris buffer at pH 7.4 containing 1 mM EDTA, 0.1% lauryl maltoside, 100 μM 5-DRF, and 100 mM KCl. The sample cuvette was degassed and subjected to a N_2 dye laser (BBQ at 390 nm) flash. The signals are sums of four flashes normalized to one. (A) Reduction of cytochrome *c* by flavin semiquinone and reoxidation of ferrocytochrome *c* were followed at 550 nm. (B) Reduction of cytochrome *a* by the ferrocytochrome *c* was observed at 604 nm. Both transients were fitted to a sum of two exponentials (solid curves).

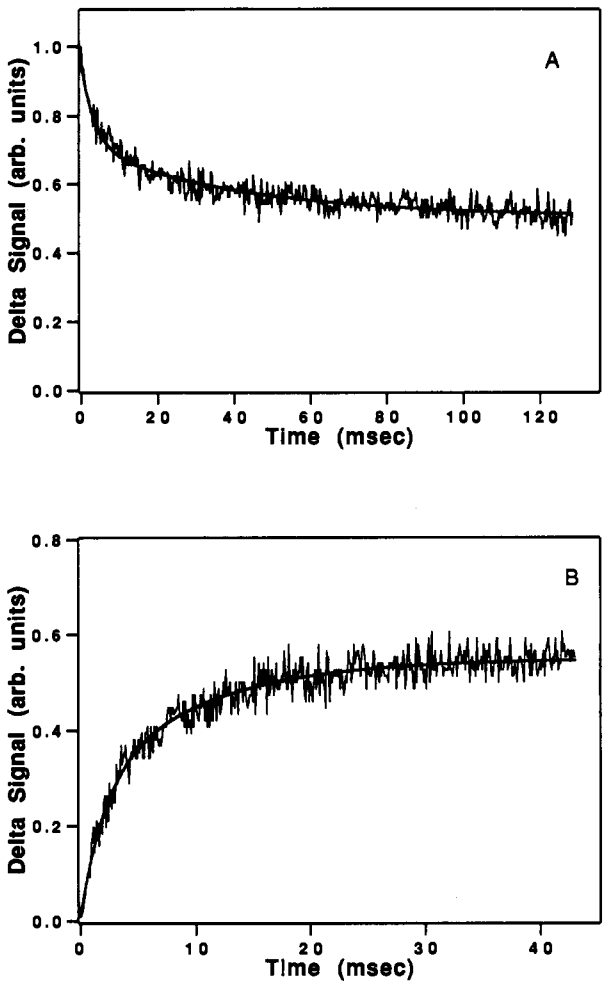

Figure 2. Intracomplex ET between cytochrome *c* reduced by photogenerated flavin semiquinone and Cu_A-depleted CcO. The experimental conditions are identical with those in Figure 1. Solid curves depict double-exponential fits to the data.

both the native and Cu_A-depleted enzymes (Figure 3), suggesting that CcO reduction proceeds via a mechanism in which a 1:1 transient complex is formed between ferrocytochrome *c* and CcO:

$$\text{Cyt } c^{2+} + (\text{CcO})_{ox} \underset{k_2}{\overset{k_1}{\leftrightarrow}} \text{Cyt } c^{2+}{:}(\text{CcO})_{ox} \quad (1)$$

$$\text{Cyt } c^{2+}{:}(\text{CcO})_{ox} \xrightarrow[k_{et}]{} \text{Cyt } c^{3+} + (\text{CcO})_{red} \quad (2)$$

Thus, at sufficiently high [CcO], k_{et} becomes rate limiting. The data fit well the following expression derived for the pseudo-first-order rate constant according to this reaction mechanism:

$$k_{obsd} = k_{et}K_A[\text{CcO}]_{ox}/(K_A[\text{CcO}]_{ox} + 1) \quad (3)$$

where K_A ($=k_1/k_2$) is the association constant for the formation of the ferrocytochrome *c*:CcO complex. The best fits to the data shown in Figure 3 give the same K_A values for both the native ($K_A = 5.4 \times 10^4$ M^{-1}) and Cu_A-depleted ($K_A = 5.0 \times 10^4$ M^{-1}) CcO, indicating that the Cu_A depletion has not disrupted the complex formation. The rates of intracomplex ET, however, are appreciably different, with k_{et} values of 2580 ± 30 s^{-1} and 740 ± 12 s^{-1} for native and Cu_A-depleted enzymes, respectively.

The overall ET reaction stoichiometries in these experiments can be deduced[10] from the observed signals, which were normalized and shown in Figures 1 and 2. A 1:1 molar ratio of ferrocytochrome *c* reoxidized to cytochrome *a* reduced was obtained for both the native (1.13 μM/1.09 μM) and Cu_A-depleted (0.48 μM/0.50 μM) samples.[11] This is the expected result, since the extent of the reaction is limited by the numbers of reducing equivalents generated by the laser excitation of 5-DRF in these experiments. Only about 5% of the cytochrome *c* is reduced by the laser flash, and these reducing equivalents are rapidly consumed by CcO in single ET from the ferrocytochrome *c* to the oxidase because of the high specific activity of our enzyme (turnover number ~ 500 s^{-1}).

The results of the present study clearly indicate that Cu_A plays an important role in the initial electron-input reaction from ferrocytochrome *c* to the native CcO. Either Cu_A is the primary electron acceptor, accepting an electron directly from cytochrome *c* and then transferring it to cytochrome *a*[6] (the intramolecular ET between cytochrome *a* and Cu_A is extremely facile and not rate-limiting[12,13]), or Cu_A regulates the rate of direct ET from

(10) To calculate the reaction stoichiometries, the observed signal changes at 604 and 550 nm were converted to Δ(absorbance) by using the appropriate scaling factor, after correcting for the percent attenuation at each wavelength (26% and 50%, respectively). The concentration changes in ferrocytochrome *c* and reduced cytochrome *a* were determined by using $\Delta\epsilon_{604} = 20$ mM^{-1} cm^{-1} and $\Delta\epsilon_{550} = 22$ mM^{-1} cm^{-1}, respectively.

(11) In previous results using a different CcO preparation,[5] a 2:1 stoichiometry was obtained between cytochrome *c* reoxidation and heme *a* reduction. The reason for this discrepancy is unclear at present and deserves further study.

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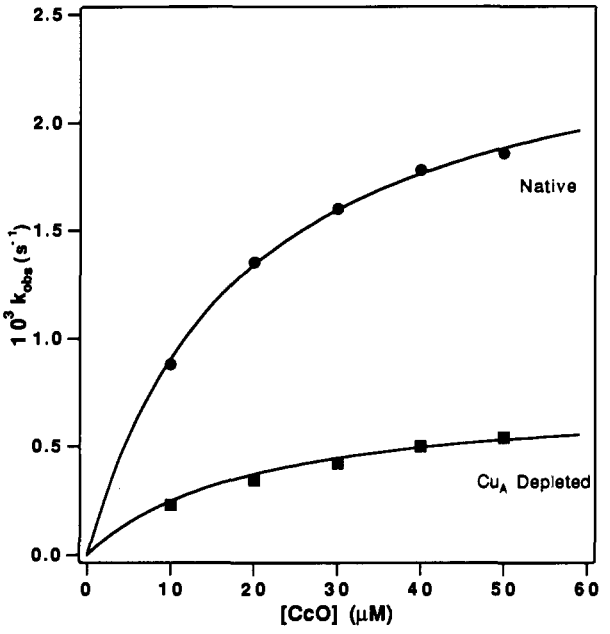

Figure 3. Kinetics of ET between ferrocytochrome *c* and CcO at various CcO concentrations and an ionic strength of 110 mM. The reaction conditions are as shown in Figure 1, and the concentration of cytochrome *c* is unchanged as the concentration of CcO is varied. The pseudo-first-order rate constants for the reduction of cytochrome *a* during the fast phase are plotted as a function of the concentration of CcO. The solid curves represent the best fits of the data to eq 3.

ferrocytochrome *c* to cytochrome *a*. Since the reduction potential of cytochrome *a* is significantly higher than that of Cu_A when the enzyme is fully oxidized, cytochrome *a* is expected to provide the ultimate disposition of the electron prior to subsequent ET to the dioxygen reduction site. Our experiments with the Cu_A-depleted enzyme show, however, that cytochrome *a* can also accept an electron directly from cytochrome *c*, albeit at a decreased kinetic rate. Thus we have the distinct possibility that there exist two distinct electron-input ports for ET from ferrocytochrome *c* to CcO, with the more facile ET proceeding via Cu_A.

New Type of Charge-Transfer Complex from an Antiaromatic Electron Donor. Possible Radical Cation Stabilization by the Captodative Effect

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One of the requirements for formation of conducting organic charge-transfer (CT) salts is the formation of resonance-stabilized radical-ion species from reaction of donor and acceptor molecules.[1] Design of suitable component molecules might incorporate aminocarboxy captodative stabilization as in the Kosower radical[2] or 3,5,5-trimethyl-2-oxomorpholinyl radical.[3] We report the synthesis and characterization of a novel electron donor for CT salts based on the 1,4-dihydropyrazine ring system, 4a,8a-diaza-2,6-dioxa-3,4,7,8-tetrahydro-4,4,8,8-tetramethylanthracene-1,5-dione (**1**, DDTTA), and its oxidation to an exceptionally persistent, captodatively stabilized radical cation. We also report

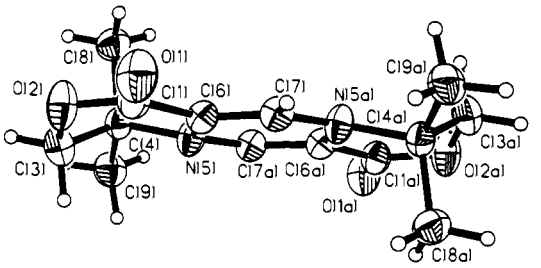
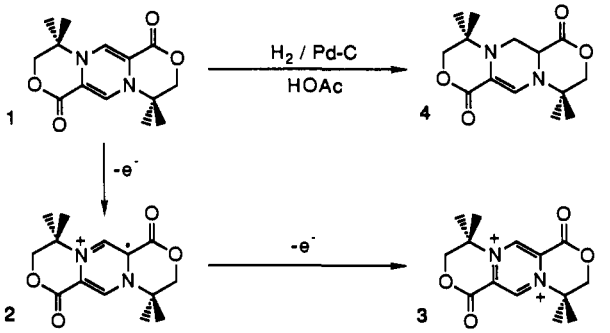


Figure 1. Thermal ellipsoid plot of **1** showing the numbering scheme adopted.

the structure and electrical properties of the complex of **1** with the electron acceptor tetracyanoquinodimethane (TCNQ).



3-(Chloromethyl)-5,6-dihydro-5,5-dimethyl-1,4-oxazin-2-one, synthesized via the method of Himmelsbach et al.[4] from 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one and *tert*-butyl hypochlorite, underwent base-promoted self-condensation in *N,N*-dimethylformamide to give DDTTA in 22% yield, ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 12 H), 3.79 (s, 4 H), 6.28 (s, 2 H). DDTTA was isolated as a bottle green, crystalline solid, soluble in a variety of solvents to give blue to blue-violet solutions. The color of **1** appears to be due to a symmetry-forbidden $\pi-\pi^*$ band in the 590–630-nm region. Both the position and extinction coefficient of this band are highly solvent dependent. Increasing the Lewis acidity of the solvent (as measured by acceptor numbers)[5] increased both the wavelength and extinction coefficient, suggesting strong coordination of the first excited state to the solvent. Such a visible absorption band has not been observed for any other stable 1,4-dihydropyrazine derivative. Though the 1,4-bis(trialkylsilyl) and 1,4-bis(trimethylgermyl) derivatives are colored (yellow and red, respectively), this was attributed to intramolecular charge transfer from the ring to low-lying orbitals at the trialkylsilyl or trialkylgermyl substituents.[6] X-ray diffraction measurements on a single crystal of **1** (Figure 1) revealed that the central ring is somewhat elongated (cf. cyclobutadiene[7] and push–pull substituted cyclobutadienes[8]) and close to planar, the mean deviation of the six ring atoms from a least-squares plane being 0.02 Å. The central ring can thus be considered to be an "antiaromatic" eight-π-electron ring system. Further evidence for this came from breaking the annular conjugation of the system. Thus, catalytic hydrogenation of **1** gave 4a,8a-diaza-2,6-dioxa-3,4,7,8,9,9a-hexahydro-4,4,8,8-tetramethylanthracene-1,5-dione (**4**), in which the remaining vinylic proton resonates at δ = 7.14 ppm. Similar paratropic shifts have been observed in other stable 1,4-dihydropyrazines;[9] such an effect is considered evidence for an "antiaromatic ring" in a single-ring system.[10]

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