in the presence of innocent tetra(*n*-butyl)ammonium, Na^+ lowers the half-wave reduction potential by 400 mV to −0.86 V and Li^+ by 600 mV to −0.67 V.

Summarizing, cyclovoltammetric and ESR/ENDOR information[9] has been gathered on triple ion intermediates along the two-step single electron transfer pathway of *p*-benzoquinone-18-crown-6 to its dihydroquinone dianion salt (eq 1) in aprotic solutions with surplus Me^+X^- salt content (cf. also ref 7). Shape-selective Me^+ encapsulation, depending on the optimum cation radius $r_{Me}{}^+$ and accompanied by drastic structural changes, has been found, together with weaker ion pairing, inversely proportional to the counter cation radius, $1/r_{Me}{}^+$. It has been pointed out before[7,11] that redox-active chelating agents capable of considerable structural changes—like the one investigated here—and their ion pairs might have biological as well as industrial implications. Presumably, this applies even more to triple ions with different binding sites for different cations, which should allow for additional fine-tuning of molecular carriers and "turn on–turn off" switches.[1]

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Oxidant-Dependent Nonadiabatic Intervalence Transitions

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We have found evidence that the reduced oxidant used to generate the mixed valence species I apparently serves as a necessary "cofactor" in the observation of an intervalence transfer (IT) band. Such metal cofactors are commonly present in redox processes in nature although their precise role often is not well understood. Our system may usefully serve as a model for such processes.

I

Compounds such as I containing two transition-metal atoms in different oxidation states often have bands in the near-infrared region of the spectrum. These light-induced transitions between the metal centers have been designated IT bands, and they are present only for the mixed-valence state.[1-6] Considerable work has been performed on these types of complexes, and much is now known about the effect of distance[7-12] between the metal centers

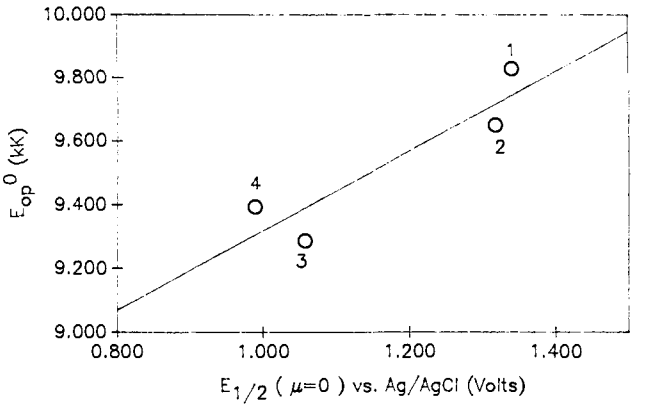

Figure 1. Oxidant potential dependence of intervalence transfer bands extrapolated to zero ionic strength. Oxidants shown are (1) $[Ru(bpy)_3]^{3+}$, (2) $[Ru(bpy)_2(py)_2]^{3+}$, (3) $[Ru(bpy)_2(CN)_2]^+$, and (4) $[Ru(bpy)_2(PPh_3)Cl]^{2+}$. Potentials were measured in DMSO by Osteryoung square wave voltammetry at a series of ionic strengths for each oxidant, and the potential at zero ionic strength was determined graphically. The range of ionic strengths employed was the same as that used in the spectroscopy experiments.

on the electron-transfer process as well as the influence of the bridging ligand or "intervening matter" on the extent of electronic coupling that is possible.[8,9,13]

The simple dielectric continuum model has been used widely[14-23] to evaluate the relative importance of inner-sphere reorganizational energies (changes in internuclear bond distances) and outer-sphere reorganizational energies (influence of solvent and ionic species in solution). Recently, it has been recognized that there are severe problems associated with the application of this model. Drickamer and Hendrickson et al.[24,25] have shown that the simple dielectric continuum model does not explain the solvent dependence of the energy band maximum of the intervalence transfer electronic absorption band (E_{op}) since only small changes in the energy of the IT band were seen for solutions of mixed-valence complexes when pressure-induced freezing was employed to dramatically vary D_s. The same group later showed[26] that there is a strong concentration dependence of E_{op} for mixed-valence biferrocenium triiodide dissolved in either nitrobenzene or dichloromethane. This was assigned to the effects of ion aggregation, and it was concluded that the increase in E_{op} with increasing concentration reflects an increasing percentage of ion-paired mixed-valence cations that have a higher energy IT band than the non-ion-paired cation because the ion pairing probably introduces a zero-point energy separation between the two vibronic states of the cation. We also recently have demonstrated[27] that there is an ionic strength dependence on the value of E_{op} obtained in dimethyl sulfoxide and *N*-methylformamide for μ-2,6-dithiaspiro[3.3]heptanedecaamminediruthenium(II,III) (complex I). At zero ionic strength, the values of E_{op} were identical, within experimental error, in the

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two solvents. Electron transfer within this molecule is known to be significantly nonadiabatic, and tunneling is an important mechanism.

Figure 1 shows the dependence of E_{op} on the reduction potential of the oxidant used to generate the mixed-valence species I from the fully reduced (II,II) complex. As before,[27] in order to observe the correlation, it was necessary to measure the ionic strength dependence of each oxidant and extrapolate the line so obtained to the value at zero ionic strength ($E_{op}°$). All ruthenium complexes were isolated as the NO_3^- salts, and NH_4NO_3 was employed to adjust the ionic strength. The solvent was dimethyl sulfoxide.

There are two mechanisms by which a dependence of the E_{op} value on the potential of the incoming oxidant may be rationalized. This depends on whether the reduced oxidant is interacting symmetrically or unsymmetrically with the two metal ions in the mixed-valence species. If it is interacting symmetrically, then the actual shape of the potential energy wells must be affected by this interaction, the depth of the wells being dependent upon the potential of the oxidant employed (or its charge since the potential and charge of the oxidant molecules also correlate with each other). If the interaction is unsymmetrical, then the observed shift in E_{op} as a function of the oxidant employed may result from a zero-point energy difference which changes in response to the oxidant present.

In either case, the complexes must exist as a solvent-caged ion aggregate long enough for the reduced oxidant to modify favorably the molecular orbitals involved in transferring the electron in the binuclear. Conductance measurements showed a strong ion aggregation between the oxidant and the reduced binuclear which is surprising in view of the 7+ overall charge of this aggregate.

We are further exploring these ideas especially with regard to more strongly electronically coupled systems to see if this work is, in fact, symptomatic of a more general problem, or if this behavior will be displayed only by systems which are markedly nonadiabatic, as is true of most biological redox processes.

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Oxidative Cyclization Chemistry Catalyzed by Clavaminate Synthase

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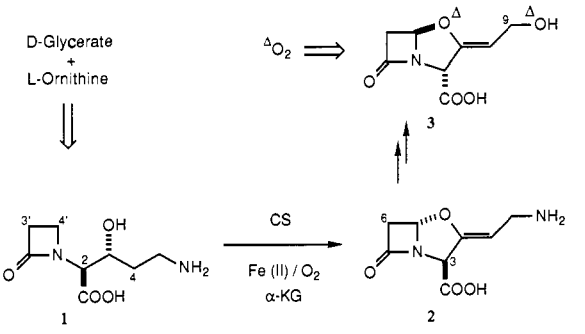
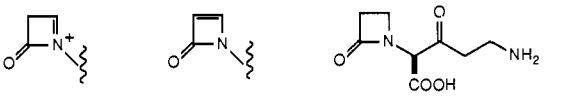
Clavaminate synthase plays a central role in the biosynthesis of the potent lactamase inhibitor clavulanic acid (**3**). This enzyme has been purified to homogeneity and found to carry out the double oxidative cyclization of proclavaminic acid (**1**) to clavaminic acid (**2**) in the presence of ferrous ion, dioxygen, and α-ketoglutaric acid.[1,2] In this communication we describe the first mechanistic studies of this enzyme which indicate that conventional hydroxylase activity has been replaced by oxidative cyclization chemistry having parallels to biochemical sulfur introduction[3] characteristic of e.g., biotin, lipoate, penicillin, and cephalosporin biosynthesis.

The oxazolidine oxygen of clavulanic acid (**3**) has been established to be derived from molecular oxygen ($^{\Delta}O_2$, Scheme I).[4] The dioxygenase cofactors of clavaminate synthase (CS) suggest

Scheme I



that the ring oxygen could be introduced at this stage from molecular oxygen. Alternatively, this oxygen could be retained from an earlier ornithyl hydroxylation step leading to the formation of proclavaminic acid (**1**). In the course of the CS reaction, oxidation takes place at C-4′, that, a priori, could be visualized in terms of either one-electron or two-electron processes. A subset of the latter mechanistic possibilities would invoke the intermediacy of an acylimine **4** leading to cyclization with a C-3 alcohol or enolate in a 5-*endo*-TRIG sense[5] or, alternatively, in a 5-*exo*-TRIG[6] fashion through the intervention of an azetinone **5**.



Furthermore, in the course of the CS reaction, C-3 of proclavaminate attains the oxidation state of a ketone. These mechanistic possibilities involve formal loss and/or potential exchange of hydrogen at C-2 and C-3′ in addition to the obvious losses at C-3, C-4, and C-4′. Moreover, apart from proton loss through enolization, a 3-keto intermediate, e.g., **6**, may undergo oxygen exchange prior to formation of clavaminic acid.

The potential loss or exchange of hydrogen at C-2 and C-3′ in proclavaminate was examined in two experiments. [2-^{2}H,3-^{13}C]Proclavaminic acid (**11**, prepared by condensation of glycyl β-lactam **7**[7] after deuterium exchange and aldehyde **9**, which was available from [1-^{13}C]-β-alanine[4])[8,9] was combined with [3-^{13}C]proclavaminic acid and purified by preparative HPLC. Two enhanced resonances in a 1:1.2 ratio were observed in the $^{13}C\{^1H\}$ NMR spectrum (0.61 Hz resolution) of this mixture at δ 69.58 and δ 69.51 (Δδ 0.07 ppm), the upfield signal owing to a β-deuterium isotopic shift.[10,11] Incubation[12] of this labeled mixture with purified CS[2] gave a sample of clavaminic acid whose C-2 resonance was significantly enhanced above natural abundance and showed a pair of signals at δ 159.35 and δ 159.32 (Δδ 0.03

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(12) Stirred reactions at ambient temperature contained in a volume of 2.5 mL; 25 mM MOPS buffer pH 7.0, 0.5 mM dithiothreitol, 0.1 mM ascorbate, 0.01 mM ferrous ammonium sulfate, 5 mM α-KG, and 3 mM proclavaminate. Enzyme (0.5 mg) was added in two portions over a 6-h incubation period and removed by ultrafiltration prior to analysis.

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