cyclohexanones—even by a relatively bulky reducing agent such as an alkoxysiliconate ion.[15] The relatively greater yield of cis product from **2** compared to **1** is believed to be due, in part, to axial reduction of the small amount of the higher energy substrate conformer with the methyl group in an axial position.[3a]

The gas-phase stereochemical results for ketones **1–3** are generally consistent with the reported behavior of these substrates toward common reducing agents in solution such as $LiAlH_4$ and $NaBH_4$[1-3] and with the predicted diastereoselectivities for reduction by LiH obtained from MO calculations[2i] (Table I). The occurrence of this same diastereoselectivity in the gas phase implies that extrinsic factors such as specific solvation, ion-pairing, and/or metal ion coordination effects need not necessarily be invoked, i.e., that it is properly ascribed to intrinsic properties of the isolated reactants. Experiments with other cyclic, bicyclic, and acyclic ketones and ketones bearing polar substituents are in progress.

Acknowledgment. We are grateful to the National Science Foundation (CHE-8815502) and the Department of Energy, Office of Basic Energy Science for their support of this research.

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Electron-Transfer Communication between Redox-Functionalized Polymers and the Active Center of the Enzyme Glutathione Reductase

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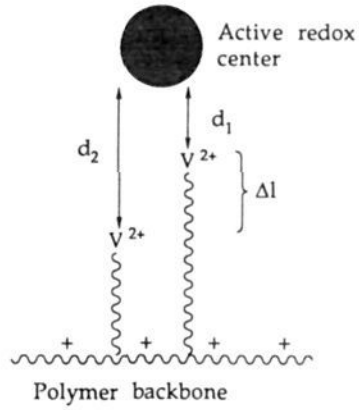
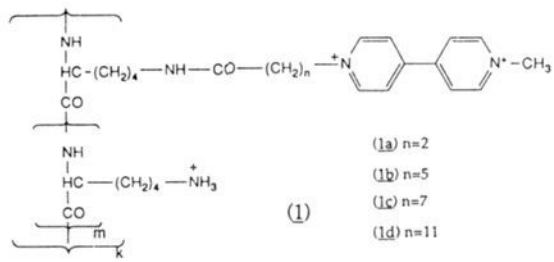
Electron-transfer (ET) processes in protein assemblies are a subject of extensive experimental[1,2] and theoretical research.[3,4] The distance,[5] stereochemical dynamics,[6] and nature of chemical bonds[7] of donor–acceptor pairs in proteins have been found to affect intra- and interprotein electron-transfer rates. ET processes between protein redox centers and their macroscopic environments are also of practical importance.[8] ET communication between proteins and electrodes is the basis for amperometric biosensors,[8] and electrical interactions between proteins and an excited species provide routes for photosynthetic transformations.[9] Recent experiments by Heller and co-workers have revealed that electrical communication between the enzyme glucose oxidase and electrodes is maintained by chemical modification of the protein with electron relay components[10] or by its interaction with redox-functionalized polymers.[11] In the latter system, electrical communication is improved as the chain anchoring the relay component to the polymer is lengthened. This has been attributed to the capability of longer chain relay components to attain closer distances to the protein active site and consequently enhance electron-transfer communication. Recent studies have revealed that the enzyme glutathione reductase, GTR, does not electrically communicate with a short-chain bipyridinium–acrylamide polymer,[12] but effective electrical wiring of the protein is accomplished by its chemical modification with bipyridinium relay components.[13] Here we wish to report on the ET processes occurring in assemblies composed of glutathione reductase with *N*-methyl-*N'*-(carboxyalkyl)-4,4'-bipyridinium-modified poly(L-lysine) (**1**). We reveal that the effectiveness of ET from the redox polymer to the protein active site is controlled by the alkyl chain length anchoring the bipyridinium salt to the polymer backbone and correlates ET rate constants with the average distance between the relay site and protein redox center.

Scheme I. Schematic Model for Spatial Orientation of Redox Functionalized Poly(L-lysine) in Respect to the Redox Site of the Enzyme





Poly[(((*N*-methyl-4,4'-bipyridinium-*N'*-yl)alkyl)carbonyl)-L-lysine] (**1**) was prepared with an average loading corresponding to 1:(68 ± 5). The kinetics of ET from the redox polymer to the redox center of GTR was followed by time-resolved laser flash photolysis in a photosystem composed of an aqueous solution, pH 7.0, containing tris(bipyridine)ruthenium(II), $Ru(bpy)_3^{2+}$, as photosensitizer, the polymer **1** as primary electron acceptor, EDTA

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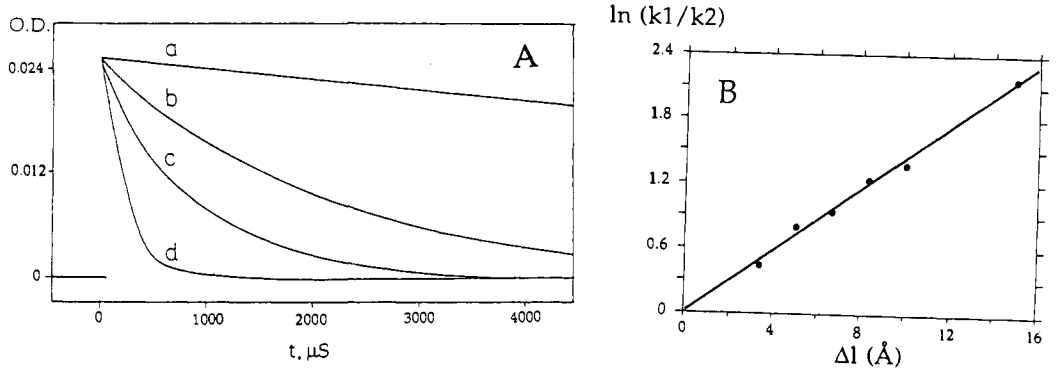

Figure 1 (A) Transient absorption spectra of photogenerated bipyridinium radical cations anchored to poly(L-lysine) (λ = 602 nm) in the absence (a) and in the presence (b–d) of the enzyme glutathione reductase. In all systems an aqueous Tris buffer solution, pH 7.0, that includes $[Ru(bpy)_3^{2+}] = 3.75 \times 10^{-4}$ M, $[EDTA] = 10^{-2}$ M, and 5.2 mg mL^{-1} (1.2×10^{-4} M) of redox-functionalized polymer was used. For curves b–d the enzyme glutathione reductase, 500 units (5.6×10^{-6} M), and $[GSSG] = 10^{-2}$ M were added: (a) includes **1d**; (b) includes **1a**; (c) includes **1b**; (d) includes **1d**. (B) Kinetic analysis of ET rate constants as a function of the difference in chain lengths, Δl, of bipyridinium components linked to the polymer.

as a sacrificial electron donor, the enzyme glutathione reductase (500 units, corresponding to 5.6×10^{-6} M), and oxidized glutathione, GSSG. In the absence of the enzyme, photolysis of the deaerated system leads to steady-state accumulation of the bipyridinium radical cation anchored to the polymer, $V^{\bullet+}$-P, by the sequence of reactions outlined in eqs 1 and 2. In the presence

$$*Ru(bpy)_3^{2+} + V^{2+}\text{-P} \rightarrow Ru(bpy)_3^{3+} + V^{\bullet+}\text{-P} \quad (1)$$

$$Ru(bpy)_3^{3+} + EDTA \rightarrow Ru(bpy)_3^{2+} + EDTA^{\bullet+} \rightarrow \text{decomposition products} \quad (2)$$

of the enzyme and substrate, decay of the photogenerated $V^{\bullet+}$-P is observed (Figure 1A). The decay of $V^{\bullet+}$-P is attributed to ET to the protein active center, eq 3, where reduction of the substrate proceeds (eq 4). Evidently, the rate of ET to the protein redox center is strongly influenced by the alkyl chain bridging the bipyridinium component to the polylysine residue, and as the bridging chain is longer, faster ET rates to the protein are observed.[14] For example, for polymers **1a–d**, the derived ET rate constants correspond to 490, 1090, 1700, and 4350 (±5%) s^{-1}, respectively.

$$V^{\bullet+}\text{-P}\cdots E_{ox} \xrightarrow{k_a} V^{2+}\text{-P}\cdots E_{red} \quad (3)$$

$$E_{red} + \tfrac{1}{2}GSSG + H^+ \rightarrow E_{ox} + GSH \quad (4)$$

To account for these different ET rates for the various redox polymers, the model outlined schematically in Scheme I has been formulated.[15] The poly(L-lysine), being positively charged, attracts the negatively charged protein ($pK_1 = 6.4$)[16] and electrically repels the positively charged bipyridinium units linked to the polymer backbone. Consequently, a macromolecular assembly is formed, where the bipyridinium redox units are in a stretched position in respect to the polymer backbone. Thus, the average distances of the various bridged bipyridinium units relative to the active site of the enzyme are determined by the alkyl chain bridging the electron mediator to the polymer. According to Marcus theory and the suggested model, the ratio of any two rate constants originating from two electron mediators positioned at different average distances in respect to the enzyme redox site will be given by eq 5, where k_1 and k_2 are the ET rate constants from the relays positioned at distances d_1 and d_2, respectively, Δl is the difference in the chain lengths linking the two relays to the polymer, and β is the electron tunneling constant. Figure 1B shows the analysis

$$\ln k_1/k_2 = \beta(d_2 - d_1) = \beta\Delta l \quad (5)$$

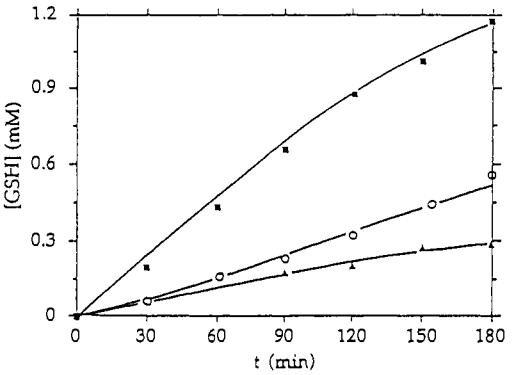

Figure 2. Rate of reduced glutathione, GSH, formation as a function of illumination time utilizing the various redox-functionalized polymers. All systems were composed of an aqueous Tris buffer solution, pH 7.0, that included $[Ru(bpy)_3^{2+}] = 7.5 \times 10^{-5}$ M, $[EDTA] = 10^{-2}$ M, glutathione reductase, 50 units (5.6×10^{-7} M), $[GSSG] = 10^{-2}$ M, and the redox polymer **1**, 3.5 mg mL^{-1} (8.1×10^{-5} M): (▲) system including **1a**; (○) system including **1b**; (■) system including **1d**.

of the experimental rate constants from the various bipyridinium bridged polymers (**1**) to the enzyme redox center. It is evident that the expected linear relationship is obtained. This implies that ET communication between the redox polymer and the active center of the enzyme is controlled by the length of the "arms" bridging the electron-transfer mediator. Longer bridging arms allow the redox mediator to attain a closer distance to the active site, thereby improving mutual electrical interactions.

The effect of the bridging arms on electrical communication with the redox center of GTR is reflected in the steady-state, photoinduced biocatalytic performance of the photosystem (Figure 2). Evidently, the rate of reduced glutathione (GSH) formation in the system follows the rate constants of electrical communication, and the highest rate of GSH formation is observed with the long-chain bridged bipyridinium polymer, **1d**.[17] We thus conclude that, within an assembly composed of the redox polymer (**1**) and glutathione reductase, ET is assisted by flexible long bridging chains attaining closer distances to the redox center of the enzyme. This reasonable intuitive phenomenon is now quantitatively supported by correlating ET rate constants and the geometrical length of the bridging arms anchoring the ET mediator.

(14) Rate constants were derived by numerical analysis of the decay curves to find the best exponential fit by the least-squares fitting routine.

(15) The decays are independent of enzyme concentrations and follow first-order kinetics in the range 50–500 units ((5.6×10^{-7})–(5.6×10^{-6}) M) of enzyme, implying the formation of a polymer–enzyme macromolecular assembly.

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(17) The ratios of steady-state photoreduction rates of GSSG by (**1**)-poly(L-lysine) do not coincide with the ratios of ET rate constants observed by transient spectroscopy and are slightly lower. This is due to the partial consumption of photogenerated GSH acting as sacrificial electron donor for the photosystem and to the fact that GSH induces product inhibition on the enzyme. cf. Scott, E. M.; Duncan, I. W.; Ekstrand, V. *J. Biol. Chem.* **1963**, *238*, 3928.

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Mediated Electron Transfer in Glutathione Reductase Organized in Self-Assembled Monolayers on Au Electrodes

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Electrical communication of enzyme redox sites and electrodes is the basis for numerous amperometric biosensor devices.[1,2] Immobilization of redox enzymes in functionalized redox polymers[3] or chemical modification of proteins with electron-transfer mediators[4] provides general means to establish electrical interactions between insulated enzyme redox centers and electrodes. Most of the presently developed, electrically communicated protein assemblies were utilized in an oxidative pathway. We have recently shown that immobilization of enzymes in bipyridinium-functionalized polymers[3d,5] or functionalization of proteins by bipyridinium components[6] establishes electrically wired biocatalytic assemblies in reductive routes. Here we wish to report on the novel development of electrically communicated enzyme electrodes by organization of monolayer redox-functionalized enzymes on Au electrodes. We describe the stepwise construction of these electrodes and reveal that the spatial structure of the redox functionalization controls the effectiveness of the electron-transfer-mediated process.

The enzyme glutathione reductase (EC 1.6.4.2) has been covalently attached[7,8] to a cysteic acid active ester monolayer that is chemisorbed to an Au electrode, according to Scheme I. The resulting electrode-immobilized protein has been treated by *N*-methyl-*N'*-(carboxyalkyl)-4,4'-bipyridinium (**1**) in the presence of urea[9] to yield an electron relay modified enzyme exhibiting electrical communication with the electrode.

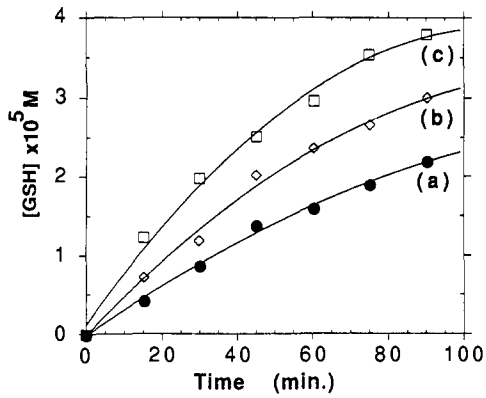
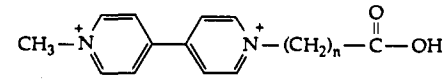


Figure 1. Rate of reduced glutathione, GSH, formation using the monolayer enzyme electrodes with different bipyridinium relays. The different curves correspond to bipyridinium units anchored by various spacers to the enzyme: (a) **1a**; (b) **1b**; (c) **1c**. All experiments were performed in a phosphate buffer, pH 7.2, and the applied potential on the working modified electrode was $E^0 = -0.72$ vs SCE; [GSSG] = 1×10^{-2} M.



(1)

(a) n=2
(b) n=5
(c) n=11

The density of active ester spacer groups associated with the Au electrode monolayer formed after step i (Scheme I) was determined to be 8×10^{-11} mol cm^{-2} by derivatization of the modified electrode with aminonaphthoquinone.[10] The density of enzyme molecules associated with the electrode was determined to be 2×10^{-11} mol cm^{-2} by immobilization of radioactive labeled glutathione reductase[11] to the electrode. The activities of bipyridinium-modified glutathione reductase ((**1a-c**)-gluthathione reductase) and of nonmodified enzyme in similar monolayer configurations on Au electrodes are identical, as revealed by the similar performances of all electrodes in the presence of solubilized methyl viologen radical as reductant. Glutathione reductase modified by **1b** or **1c** exhibits reversible cyclic voltammograms[12] ($E^0 = -0.58$ V vs SCE; $\Delta E_p = 30$ mV at $\nu < 1$ V s^{-1}). By assuming that all bipyridinium units anchored to the protein backbone are reduced in the voltammetric cycle, the charge passed in the reduction wave allows us to determine the average loading degree of the protein backbone by the redox component to be ca. 8. Namely, out of the 39 lysine residues present in the glutathione reductase backbone,[13] 8 are modified by the bipyridinium redox groups. The rate constants[14] for electron transfer from the electrode to **1b** or **1c** units anchored to the protein are similar, $k_{et} = 130$ s^{-1}.

The bipyridinium-modified enzyme monolayer electrodes were examined as biocatalytic redox assemblies for electroreduction of oxidized glutathione, GSSG (Figure 1). The active site of glutathione reductase consists of a disulfide bond formed by two cysteine residues. Its reduction potential corresponded to $E^0 =$

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(11) [^{3}H]Iodoacetic acid was anchored to cysteine residues of glutathione reductase by reacting the enzyme with an excess of the radioactive label in phosphate buffer, pH 7.3, at room temperature for 1 h followed by Sephadex G-25 separation of the enzyme.

(12) No electrochemical response in the cyclic voltammogram of the **1a**-enzyme-modified electrode is observed. However, **1a** linkage to the enzyme is detectable by the blue coloration of the Au electrode upon application of negative potential. Modification of the enzyme with **1a** is also essential to electrically wire the protein toward the reduction process.

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