Chiral Recognition in Mediated Electron Transfer in Redox Proteins

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Chiral recognition of substrate binding processes is a subject of theoretical and practical interest. Chiral recognition of substrates to chiral receptor molecules such as cyclodextrins,[1] crown ethers,[2] and cavitands[3] has been studied extensively. Chiral recognition was also observed with macromolecular systems such as polymers[4] and chiral surfaces.[5] The resulting chiroselective binding processes were widely applied in chiral separations.[6] The most prominent chiral recognition processes are observed with enzymes, where chiral discrimination of binding of substrates (or inhibitors) to the enzyme active site takes place.[7] Chiral discrimination in the association of optically-active transition-metal complexes to DNA was also observed,[8] as well as chiral discrimination in electron transfer in DNA.[9] As the primary step of ligand association to proteins is substantially slower than a diffusion-controlled process ($k \cong 10^7-10^8$ $M^{-1} \cdot s^{-1}$),[10] chiral discrimination along the substrate binding pathway is anticipated to play a role in the substrate association process. Chiroselective energy transfer with a ligand that does not bind to the enzyme active site was detected,[11] and was rationalized in terms of chiral interactions of the acceptor along its dynamic penetration pathway in the protein.

Electron-transfer mediators provide a means to establish electrical communication between redox proteins and electrode surfaces.[12] Ferrocene derivatives were widely applied as electron-transfer mediators for flavin-containing oxidoreductases such as glucose oxidase (GOx)[13] or glutathione reductase (GR).[14] Here we wish to report on the chiroselective electron transfer in GOx and GR using (*S*)- and (*R*)-*N*,*N*-dimethyl-1-ferrocenylethylamine [(*S*)- and (*R*)-Fc, Scheme 1]. The observed chiral recognition provides a means to probe the mechanism of mediated electron transfer in the proteins.

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Scheme 1. Electrobiocatalyzed Oxidation of Glucose or NADPH by GOx or GR Using Chiral Ferrocene Electron Mediators

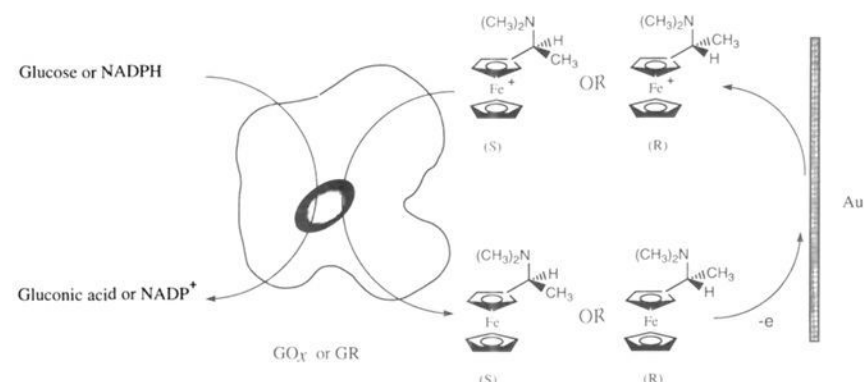

Figure 1 shows the electrocatalytic anodic currents developed in systems that contain GOx as biocatalyst and (*S*)- or (*R*)-Fc as diffusional electron-transfer mediators in the presence of glucose, 0.05 M.[15] Clearly, the (*S*)-Fc acts as a superior electron-transfer mediator as compared to (*R*)-Fc, and the electrocatalytic anodic current is ~2-fold higher. Figure 2a shows the electrocatalytic anodic currents in the two systems at different concentrations of the electron transfer mediators. Similar saturation currents are observed in the two systems, but the I_{max} value is reached at lower concentrations of the (*S*)-Fc as compared to the (*R*)-Fc.[16] The electrocatalytic anodic currents were analyzed in terms of the Michaelis–Menten model. Figure 2b shows the Lineweaver–Burk plots for the (*S*)- and (*R*)-Fc systems. The value of $I_{max} = 25$ μA is identical in the two systems, but the K_m values differ substantially: $K_m^{(S)\text{-Fc}} = 0.04$ mM, $K_m^{(R)\text{-Fc}} = 0.11$ mM. This kinetic analysis is analogous to a noncompetitive inhibition pathway of the enzyme. Since the I_{max} values for the two electron mediators, (*S*)- and (*R*)-Fc, are similar, the rates of electron transfer from the FAD cofactor to the ferrocenyl cation enantiomers are identical. Thus, the different K_m values obtained for the enantiomers imply that the ratio of association and dissociation rates of the electron-transfer mediators is controlled by chiral interactions with the protein. As no specific binding site for the electron-transfer mediators exists in the protein, the rates of association and dissociation represent the dynamics of penetration of the electron mediator within the protein to reach the appropriate distance from the FAD cofactor that allows electrical communication. The kinetic analysis suggests that chiral interactions between the electron-transfer mediators and the protein along the diffusion pathway provide dynamic control for the formation of the FAD–ferrocenyl cation pair that facilitates electron transfer.

Chiral recognition was also examined in the electron-transfer process involving the GR-biocatalyzed oxidation of NADPH using (*S*)- and (*R*)-Fc as electron-transfer mediators.[15] Figure 3 shows the electrocatalytic anodic currents developed upon biocatalyzed oxidation of NADPH by (*S*)- and (*R*)-Fc, respec-

(15) All the experiments were carried out in a three-compartment electrochemical cell, using a gold wire as the working electrode (geometrical area, 0.2 cm^2; roughness factor, 1.2), a graphite auxiliary electrode isolated by a glass frit, and a saturated calomel electrode (SCE) connected to the working volume with a Luggin capillary as reference electrode. The electrochemical experiments were performed by mixing 100 units of the enzyme GOx (type X-S from *Aspergillus niger*, EC 1.1.3.4) or GR (type III from bakers' yeast, EC 1.6.4.2) with the respective enzyme substrate (at saturation conditions): 50 mM glucose or 5 mM NADPH for GOx and GR, respectively, in a phosphate buffer solution, 0.01 M, pH = 7.0, containing 0.1 M Na_2SO_4. (*S*)- and (*R*)-Fc were injected to generate the appropriate concentration using an ethanolic stock solution of the electron mediators. All experiments were performed under inert Ar atmosphere.

(16) The (*R*)- and (*S*)-Fc exhibit identical cyclic voltammograms in the presence of GOx and in the absence of glucose, implying similar diffusion coefficients of the electron mediators. The results described are highly reproducible, and in a series of three experiments, independent of the electron mediator used, identical results were obtained. The real surface areas of the Au electrodes were determined electrochemically prior to each experiment (cf.: Woods, R. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1980; Vol. 9, pp 1–162).

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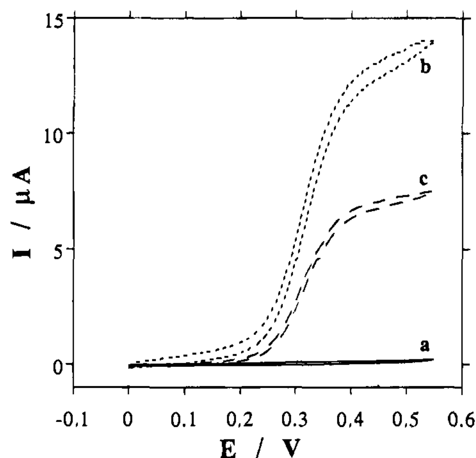

Figure 1. Cyclic voltammograms of (a) background solutions of systems consisting of GOx (100 units) in the presence of (*R*)-Fc, 4×10^{-5} M; (b) system containing GOx (100 units), (*S*)-Fc, 4×10^{-5} M, in the presence of glucose, 0.05 M; and (c) system composed of GOx (100 units), (*R*)-Fc, 4×10^{-5} M, and glucose, 0.05 M. Scan rate for all systems is 2 mV·s^{-1}.

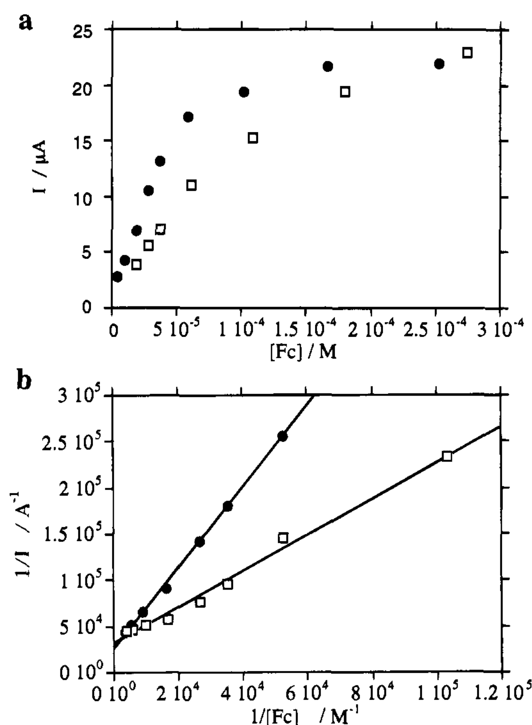

Figure 2. (a) Electrocatalytic anodic current as a function of electron mediator concentration observed in systems that include GOx (100 units) and glucose, 0.05 M. (●) In the presence of (*S*)-Fc. (□) In the presence of (*R*)-Fc. Measurements were performed at 25 ± 0.5 °C. (b) Lineweaver–Burk plots of the dependence of the electrocatalytic anodic currents on electron mediator concentrations.

tively. In contrast to the previous system, here (*R*)-Fc acts as a superior electron-transfer mediator as compared to the (*S*)-Fc electron relay. The electrocatalytic anodic current in the presence of (*R*)-Fc is higher by ~15% as compared to that of the system that includes (*S*)-Fc. The electrocatalytic anodic currents were recorded at different concentrations of (*R*)- and (*S*)-Fc, and the results were analyzed in terms of the Michaelis–Menten model, as described before. In contrast to the previous system, we find that the K_m values for (*R*)- and (*S*)-Fc are

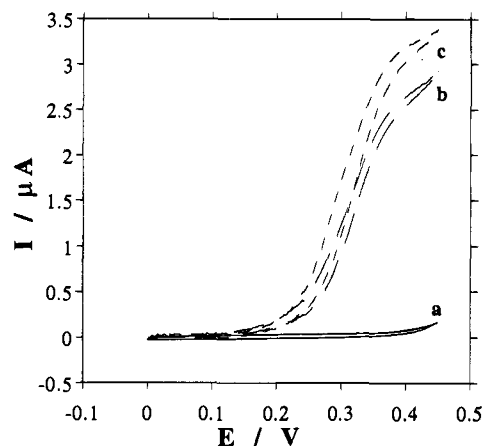

Figure 3. Cyclic voltammograms of (a) background solution of systems consisting of GR (100 units) in the presence of NADPH, 5×10^{-3} M; (b) system composed of GR (100 units), NADPH 5×10^{-3} M, and (*S*)-Fc, 6×10^{-5} M; and (c) system composed of GR (100 units), NADPH, 5×10^{-3} M, and (*R*)-Fc, 6×10^{-5} M. Measurements were performed at 25 ± 0.5 °C.

identical, $K_m^{(R)\text{-Fc}} = K_m^{(S)\text{-Fc}} = 0.4$ mM, while the V_{max} values of the two systems differ, $I_{max}^{(R)\text{-Fc}} = 23.5$ µA, $I_{max}^{(S)\text{-Fc}} = 21$ µA. Thus, the chiral recognition in the biocatalyzed oxidation of NADPH is not controlled by the affinities of association of the electron mediators to the protein, but rather originates from the electron-transfer process itself. The difference in the mechanisms of chiral recognition in the two flavin-containing enzymes can be attributed to the location of the FAD cofactor units in the protein assemblies. In GOx the two FAD units are embedded inside the protein,[17] whereas in GR the FAD units are positioned at the exterior protein periphery.[18] Thus, for GOx, diastereoisomeric interactions between the chiral electron mediators and the protein exist along the penetration path of the relays to the embedded FAD sites. This is reflected by the different associative affinities between the chiral electron mediators and GOx, resulting in the chiral discrimination in the biocatalyzed oxidation of glucose. For GR, the diastereoisomeric interaction pathway of the electron mediators with the protein is negligibly short, and hence their association affinities are similar. The chiral discrimination in the electrobiocatalyzed oxidation of NADPH is then dominated by the diastereoisomeric relation of the Fc electron mediator and the protein complex to the FAD sites. The orientation of the electron mediators in the diastereoisomer structures relative to the FAD sites might lead to the observed chiral discrimination.

In conclusion, we have demonstrated that chiral recognition plays an important role in electron transfer in redox enzymes. Analysis of the electrobiocatalyzed oxidation of glucose and NADPH by (*S*)- and (*R*)-Fc, using GOx and GR, respectively, allowed us to reveal that chiral recognition could originate from diastereoisomeric interactions accompanying the electron mediator penetration into the protein, as well as from the diastereoisomeric properties of the resulting electron mediator–enzyme complex.

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