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Electrochemical Fixation of Carbon Dioxide in Oxoglutaric Acid Using an Enzyme as an Electrocatalyst

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We report the first electrochemical fixation of CO_2 in oxoglutaric acid using an enzyme (isocitrate dehydrogenase, ICDH) as an electrocatalyst and methylviologen (MV^{2+}) as a mediator. The product is isocitric acid. The reaction occurs selectively with current efficiencies approaching 100% at -0.95 V vs SCE in a 0.2 M tris buffer (pH 7). These conditions are the mildest reported to date for efficient reduction of CO_2. Enzymes have previously been used as electrocatalysts for the direct reduction of CO_2 to formic acid but not for the fixation of organic compounds.[1] The principle applied here is to reverse the in vivo metabolic pathway of isocitric acid oxidation to yield oxoglutaric acid and CO_2. These are catalyzed by ICDH in the presence of nicotinamide adenine dinucleotide phosphate ($NADP^+$). Uniquely, this electrolytic system does not require the use of $NADP^+$.

To date, most studies on the electrochemical reduction of CO_2 have focused on the direct reduction of CO_2 to carbon monoxide, formic acid, formaldehyde, etc. Enhanced reaction selectivity and/or creation of milder electrolysis conditions have been reported.[2] In addition, direct reduction to methane has recently attracted much attention.[3] The efficient reduction of bicarbonate to formate has been reported[4] as CO_2-related electrochemistry. Only one example of the fixation of organic compounds has been published: the electrolysis of 1,4-benzoquinone, in the presence of CO_2, yielded 2,5-dihydrobenzoic acid.[5]

The present reaction system was constructed by postulating that when CO_2 is reductively fixed in an organic molecule, the enzyme is oxidized; the oxidized enzyme is to ultimately be reduced back to its original form by methylviologen cation radicals. The latter are produced at a glassy carbon cathode. Cyclic voltammograms taken in the presence and absence of CO_2 are shown in Figure 1. Solution conditions include 0.2 M tris buffer (pH 7.7, 0.2 M $NaHCO_3$), 1.0×10^{-4} M MV^{2+}, 1 unit of ICDH, and 1.0×10^{-2} M oxoglutaric acid. It is well-known that the cathodic current peaks at -0.75 and at -1.05 V are due to the reduction of MV^{2+} to $MV^{•+}$ and $MV^{•+}$ to MV^0, respectively. When CO_2 was introduced, these cathodic waves increased slightly, while the anodic waves were slightly suppressed. This suggests that CO_2 fixation could be accomplished with the above-postulated reaction scheme. The electrolysis experiments were carried out in a two compartment cell separated by a Nafion membrane at potentials sufficiently negative to reduce MV^{2+} to $MV^{•+}$. One compartment
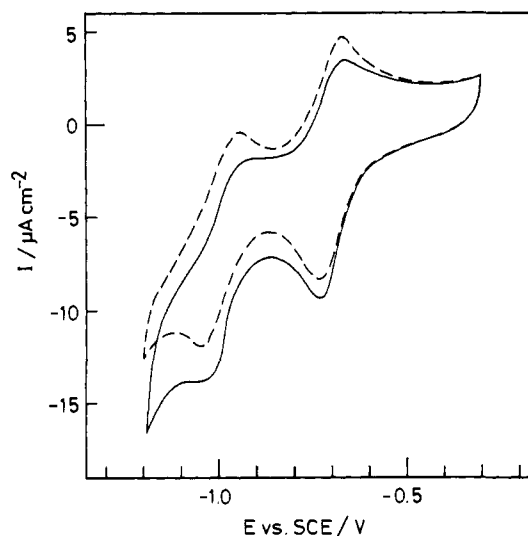

Figure 1. Cyclic voltammograms taken in 0.2 M tris buffer solutions (pH 7.7, 25 mL) containing 0.2 M $NaHCO_3$, 1.0×10^{-4} M MV^{2+}, 1 unit of ICDH, and 1.0×10^{-2} M oxoglutaric acid saturated with N_2 (--) and CO_2 (—). Sweep rate was 10 mV s^{-1}. A glassy carbon electrode was used.

Table I. Electrochemical Fixation of Carbon Dioxide in Oxoglutaric Acid To Yield Isocitric Acid[a]

E (V vs SCE)	charge (C)	C (MV^{2+}) (M)	amount produced (μmol)	current efficiency (%)	
				apparent	net
-0.75	0.88	1.0×10^{-4}	2.77	60.6	82.8
-0.85	1.53	1.0×10^{-4}	6.65	83.9	99.2
-0.95	1.65	1.0×10^{-4}	7.33	85.7	100
-0.95	1.60	5.0×10^{-5}	7.16	86.4	93.2
-0.95	1.45	2.0×10^{-5}	6.43	85.5	88.4
-0.95	0.96	1.0×10^{-5}	3.79	76.3	78.0

[a] The CO_2-saturated electrolyte (25 mL) was 0.2 M tris buffer containing 0.2 M $NaHCO_3$, 1 unit of ICDH, and 1×10^{-2} M oxoglutaric acid. Selected concentrations of MV^{2+} are given in the table.

contained 25 mL of the above described CO_2-saturated electrolyte solution to which selected concentrations of MV^{2+} were added. The other cell contained only the tris buffer. Reaction product analysis was carried out with liquid chromatography.

Results obtained are shown in Table I. Both apparent and net current efficiencies are reported. The former was obtained by applying the coulombs consumed in the electrolysis to the amount of isocitric acid produced assuming that two electrons were involved in the fixation reaction. Current efficiencies >80% were achieved in each case. In the present reaction system, not MV^{2+} but $MV^{•+}$ must be used to regenerate ICDH. Thus, a fraction of the total quantity of electricity consumed in the electrolysis can be attributed to the initial reduction of MV^{2+} to $MV^{•+}$. This quantity (0.24 C for the case of 1×10^{-4} M MV^{2+}) is subtracted from the total numbers of coulombs to give net values. Net current efficiencies were then obtained as shown in Table I. Note that 100% net current efficiency is observed for the electrolysis at -0.95 V vs SCE in the presence of 1×10^{-4} M MV^{2+}. Decreases in MV^{2+} concentration reduce current efficiencies for CO_2 fixation. No fixation products were observed in the absence of MV^{2+}, indicating that MV^{2+} worked as an efficient mediator to recycle ICDH. Isocitric acid was produced in proportion to the electrolysis charge, judging from electrolysis results at -0.95 V vs SCE. If it is assumed that 1 unit of ICDH contains 1.5×10^{-9} mol of redox centers,[6] the turnover number of ICDH in the CO_2 fixation process amounts to more than 5500 for the case of electrolysis of 2.8 C.

The results shown here are significant for several reasons. Firstly, the electrolytic system does not require the use of $NADP^+$. $NADP^+$ normally plays an important role in vivo metabolic

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systems. It should be stressed that the ICDH used in the present study contained no $NADP^+$, as judged from the lack of its characteristic UV absorption spectrum. Willner et al.[7] accomplished the same fixation reaction by using a photosensitized NADPH regeneration system. In this case $Ru(bpy)_3^{2+}$ was used as a photosensitizer, d,l-dithiothreitol as an electron donor, and ferredoxin-$NADP^+$ reductase as an enzyme to recycle NADPH. The conversion efficiencies were relatively low (4.6%). Secondly, the potential at which efficient CO_2 fixation has been accomplished is the least negative reported to date. Frese et al.[3c] previously reduced CO_2 at Ru electrodes in aqueous solutions (pH 3) at –0.35 V vs SCE. However, current efficiencies were less than 1%. Finally, the results obtained in this study suggest that a variety of CO_2 fixation reactions may be developed by using enzymes that are involved in vivo CO_2-related metabolic pathways as electrocatalysts.

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Quenching of Naphthalocyanine Triplets by O_2: Application of the Sandros Equation

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Recently we showed that the triplet (T_1) energy of bis(tri-n-hexylsiloxy)silicon 2,3-naphthalocyanine (SiNc, see below) is 1.0 kcal/mol lower than that of singlet molecular oxygen (22.5 kcal/mol).[1] Sandros[2] showed that for endergonic triplet energy transfer reactions the bimolecular rate constant decreases nearly exponentially with increasing energy gap between the donor and the acceptor.[3] Thus, small variations in the triplet energies of metal 2,3-naphthalocyanines caused by changes in the nature of the metal and solvent are expected to have a significant effect on the efficiency of triplet quenching by O_2. Further, if the S_1-T_1 energy gap (E_{ST}) remains constant within the 2,3-naphthalocyanine family, then the quenching rate constant should be related by the Sandros equation to the energy of the S_0-S_1 transition. The results described below show that the predicted relationship is obeyed with E_{ST} = 15.6 ± 0.4 kcal/mol, in agreement with the value determined spectroscopically for SiNc.[1] This is the first example of the application of the Sandros equation to triplet quenching by O_2 and the first to correlate triplet energy transfer kinetics with singlet energy levels.

The Sandros equation[2] relates the bimolecular rate constant for triplet–triplet energy transfer (k_{TT}) between a donor (D) and an acceptor (A) to the diffusion-controlled rate constant (k_{diff}) and the difference in the triplet energies (E_T) of D and A (eq 1).

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(3) This dependence of rate constant on energy gap was demonstrated concurrently by Stevens and Walker (Stevens, B.; Walker, M. S. *Proc. Roy. Soc.* **1964**, *26*, 27, 109).
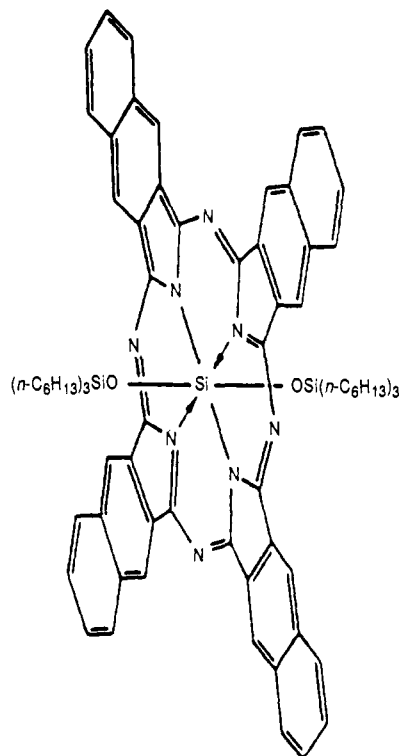
In the case of energy transfer from a donor triplet to O_2 $(^3\Sigma_g^-)$ to produce O_2 $(^1\Delta_g)$ (energy requirement E_Δ = 22.5 kcal/mol), a factor of one-ninth has to be introduced into the expression for the bimolecular rate constant $(k_{T\Sigma})$ because only one-ninth of the intervening collision complexes are of overall singlet multiplicity (eq 2). We can replace E_T by the difference between the singlet S_1 energy (E_S) and the S_1-T_1 energy gap (E_{ST}), so that the expression for $k_{T\Sigma}$ becomes eq 3.

$$k_{TT} = k_{diff}/(1 + \exp(-(E_T^D - E_T^A)/RT)) \tag{1}$$

$$k_{T\Sigma} = (^1/_9)k_{diff}/(1 + \exp(-(E_T - E_\Delta)/RT)) \tag{2}$$

$$k_{T\Sigma} = (^1/_9)k_{diff}/(1 + \exp(-(E_S - E_{ST} - E_\Delta)/RT)) \tag{3}$$

A variety of solvents were used in these experiments, so we chose to use 2′-acetonaphthone (An) as a reference compound to compensate for the effects of the solvent dependencies of k_{diff} and O_2 solubility. The triplet energy of An (59 kcal/mol) is sufficiently greater than E_Δ that the exponential term in eq 2 is negligible, giving $k_{T\Sigma}^{An} = (^1/_9)k_{diff}$.[4] Thus, the relative values of $k_{T\Sigma}$ of a naphthalocyanine (Nc) and An in any particular solvent obey eq 4.

$$k_{T\Sigma}^{Nc}/k_{T\Sigma}^{An} = 1/(1 + \exp(-(E_S - E_{ST} - E_\Delta)/RT)) \tag{4}$$

The naphthalocyanine triplets decayed biexponentially in air- and O_2-saturated solutions due to reversible energy transfer[1] and two decay components were clearly resolvable in most cases. The measured rate of the early component (γ_1) has the analytical form given in eq 5, where $k_{\Delta G}$ represents the bimolecular rate constant

$$\gamma_1 = k_{T\Sigma}[O_2] + k_{\Delta G}[Nc] \tag{5}$$

for energy transfer from O_2 $(^1\Delta_g)$ to ground-state naphthalocyanine.[1] Values of $k_{T\Sigma}^{Nc}$ were thus obtained from the dependence of γ_1 on the percent of O_2 in the saturating gas (21% or 100%).

(4) Based on an O_2 concentration in air-saturated benzene of 1.9×10^{-3} M,[5] the absolute value of $k_{T\Sigma}$ for An in benzene was $(1.5 \pm 0.1) \times 10^9$ M^{-1} s^{-1}, which is in agreement with the value expected on the basis of $k_{diff} \approx (1–2) \times 10^{10}$ M^{-1} s^{-1}.[6,7] The triplet states of An and the naphthalocyanines were produced and monitored by laser flash photolysis with 355 nm ($\approx$10 ns) light pulses from a Q-switched Nd:YAG laser for excitation. Monitoring wavelengths were 425–430 nm for An and 580–600 nm for Nc.