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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<sub>2</sub> 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<sub>2</sub>. 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.<sup>1</sup> The principle applied here is to reverse the in vivo metabolic pathway of isocitric acid oxidation to yield oxoglutaric acid and  $\text{CO}_2$ . These are catalyzed by ICDH in the presence of nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>). 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.<sup>2</sup> In addition, direct reduction to methane has recently attracted much attention.<sup>3</sup> The efficient reduction of bicarbonate to formate has been reported<sup>4</sup> 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<sub>2</sub>, yielded 2,5-dihydrobenzoic acid.<sup>5</sup>

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<sub>3</sub>),  $1.0 \times 10^{-4}$  M MV<sup>2+</sup>, 1 unit of ICDH, and  $1.0 \times 10^{-2}$ 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<sup>2+</sup> 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<sub>2</sub> 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<sup>2+</sup> to MV<sup>++</sup>. One compartment

Figure 1. Cyclic voltammograms taken in 0.2 M tris buffer solutions (pH 7.7, 25 mL) containing 0.2 M NaHCO<sub>3</sub>,  $1.0 \times 10^{-4}$  M MV<sup>2+</sup>, 1 unit of ICDH, and  $1.0 \times 10^{-2}$  M oxoglutaric acid saturated with N<sub>2</sub> (--) and  $CO_2$  (--). Sweep rate was 10 mV s<sup>-1</sup>. A glassy carbon electrode was used.

Table I. Electrochemical Fixation of Carbon Dioxide in Oxoglutaric Acid To Yield Isocitric Acida

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

<sup>a</sup> The CO<sub>2</sub>-saturated electrolyte (25 mL) was 0.2 M tris buffer containing 0.2 M NaHCO<sub>3</sub>, 1 unit of ICDH, and  $1 \times 10^{-2}$  M oxoglutaric acid. Selected concentrations of  $MV^{2+}$  are given in the table.

contained 25 mL of the above described CO<sub>2</sub>-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<sup>2+</sup> but  $MV^{\bullet+}$  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  $1 \times 10^{-4}$  M MV<sup>2+</sup>) 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  $1 \times 10^{-4}$  M MV<sup>2+</sup>. 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<sup>2+</sup> 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  $1.5 \times 10^{-9}$  mol of redox centers,<sup>6</sup> 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<sup>+</sup>. NADP<sup>+</sup> normally plays an important role in vivo metabolic

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<sup>5</sup> 0 I / јиА ст<sup>-2</sup> -10 -15 -1.0 -0.5 Evs. SCE / V

systems. It should be stressed that the ICDH used in the present study contained no NADP<sup>+</sup>, 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<sup>+</sup> 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<sub>2</sub> 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<sub>2</sub> fixation reactions may be developed by using enzymes that are involved in vivo CO2-related metabolic pathways as electrocatalysts.

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## Quenching of Naphthalocyanine Triplets by O<sub>2</sub>: 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).<sup>1</sup> Sandros<sup>2</sup> 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.<sup>3</sup> 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  $E_{ST} = 15.6 \pm 0.4$  kcal/mol, in agreement with the value determined spectroscopically for SiNc.<sup>1</sup> This is the first example of the application of the Sandros equation to triplet quenching by O<sub>2</sub> and the first to correlate triplet energy transfer kinetics with singlet energy levels.

The Sandros equation<sup>2</sup> 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).



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  $E_{\Delta} = 22.5$  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.

$$k_{\rm TT} = k_{\rm diff} / (1 + \exp(-(E_{\rm T}^{\rm D} - E_{\rm T}^{\rm A}) / RT))$$
(1)

$$k_{\rm T\Sigma} = (\frac{1}{9})k_{\rm diff} / (1 + \exp(-(E_{\rm T} - E_{\Delta})/RT))$$
(2)

$$k_{\rm T\Sigma} = (\frac{1}{9})k_{\rm diff}/(1 + \exp(-(E_{\rm S} - E_{\rm ST} - E_{\Delta})/RT)) \quad (3)$$

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  $k_{\text{diff}}$  and  $O_2$ solubility. The triplet energy of An (59 kcal/mol) is sufficiently greater than  $E_{\Delta}$  that the exponential term in eq 2 is negligible, giving  $k_{\text{T\Sigma}}^{\text{An}} = (1/9)k_{\text{diff}}$ .<sup>4</sup> Thus, the relative values of  $k_{\text{T\Sigma}}$  of a naphthalocyanine (Nc) and An in any particular solvent obey eq 4.

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

The naphthalocyanine triplets decayed biexponentially in airand O<sub>2</sub>-saturated solutions due to reversible energy transfer<sup>1</sup> and two decay components were clearly resolvable in most cases. The measured rate of the early component ( $\gamma_1$ ) has the analytical form given in eq 5, where  $k_{\Delta G}$  represents the bimolecular rate constant

$$\gamma_1 = k_{\text{T}\Sigma}[\text{O}_2] + k_{\Delta \text{G}}[\text{Nc}]$$
(5)

for energy transfer from O<sub>2</sub>  $({}^{1}\Delta_{g})$  to ground-state naphthalocyanine.<sup>1</sup> Values of  $k_{T\Sigma}^{Nc}$  were thus obtained from the dependence of  $\gamma_{1}$  on the percent of O<sub>2</sub> in the saturating gas (21% or 100%).

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<sup>(4)</sup> Based on an O<sub>2</sub> concentration in air-saturated benzene of  $1.9 \times 10^{-3}$  M,<sup>5</sup> the absolute value of  $k_{TZ}$  for An in benzene was  $(1.5 \pm 0.1) \times 10^9$  M<sup>-1</sup> s<sup>-1</sup>, which is in agreement with the value expected on the basis of  $k_{diff} = (1-2) \times 10^{10}$  M<sup>-1</sup> s<sup>-1</sup>,<sup>6.7</sup> 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.