Photochemical Fixation of Carbon Dioxide: Enzymic Photosynthesis of Malic, Aspartic, Isocitric, and Formic Acids in Artificial Media[1]

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Photosensitized regeneration of 1,4-dihydronicotinamide adenine dinucleotide phosphate (NADPH) with an artificial photosystem allows the enzymic fixation of CO_2 through carboxylation of α-oxo acids using sacrificial electron donors. Pyruvic acid is carboxylated to malic acid and α-oxoglutaric acid is carboxylated to isocitric acid with the malic enzyme and isocitrate dehydrogenase (ICDH) as biocatalysts, $\varphi = 1.9\%$. Malic acid formed through the photosensitized process is used as a synthetic building block for subsequent sesquestered enzymic transformations, and its conversion into aspartic acid is accomplished with fumarase and aspartase as biocatalysts.

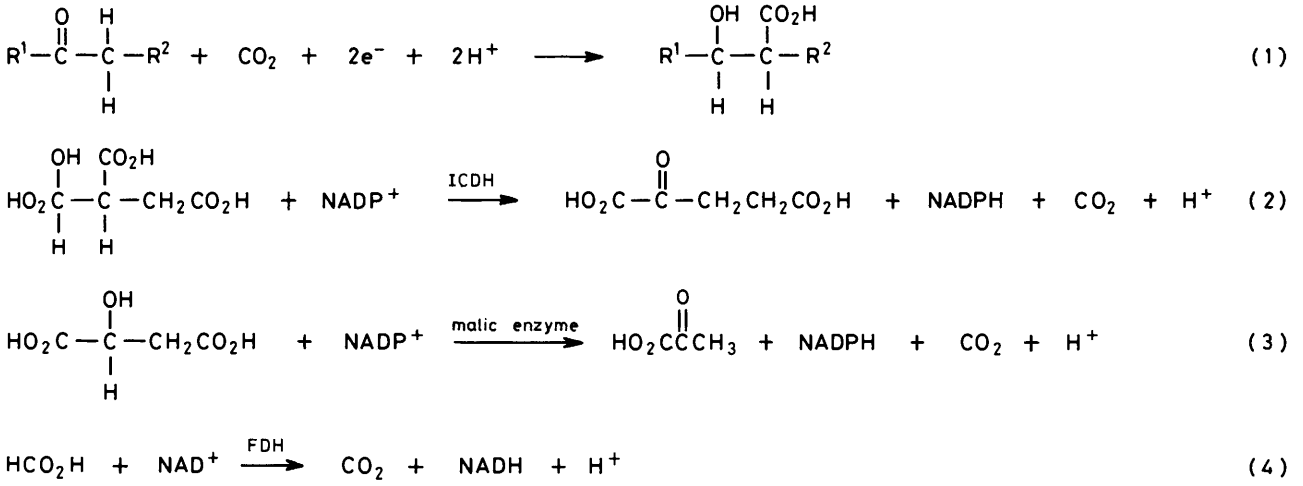
Photoreduction of CO_2 to formate is accomplished in the presence of formate dehydrogenase (FDH) as catalyst. Photosensitized reduction of different bipyridinium relay systems, *i.e.* *N,N'*-dimethyl-4,4'-bipyridinium (MV^{2+}) (**1**), *N,N'*-dimethyl-2,2'-bipyridinium (DM^{2+}) (**2**), *N,N'*-trimethylene-2,2'-bipyridinium (DT^{2+}) (**3**), and *N,N'*-tetramethylene-2,2'-bipyridinium (DQ^{2+}) (**4**), to the corresponding radical cations yields reduced relays that act as cofactors for FDH, which mediates the reduction of CO_2 to formate. The quantum yield for formate formation is in the range $\varphi = 0.5$—1.6%

Substantial efforts have been directed in recent years towards the development of solar light conversion and storage systems.[2–4] Specific emphasis has been given to the conversion of abundant materials into fuel products. In this respect, photolysis of water to hydrogen and photoreduction of carbon dioxide to organic fuel products are of special interest.[5] Photosensitized hydrogen evolution from aqueous solutions has been reported in various systems with homogeneous[6] or heterogeneous[7,8] catalysts.

Activation of CO_2 by homogeneous transition metal complexes has been extensively studied in recent years.[9,10] Electrocatalysed reduction of CO_2 to formate, oxalate, methane, *etc.* has been observed in aqueous as well as in non-aqueous media in the presence of specific electrode materials[11] or transition metal complexes,[12,13] though the reduction potentials achieved are usually too negative for application in photochemical reduction processes. A few studies have examined photoreduction of CO_2. Lehn and his co-workers[14,15] have reported on the photoreduction of CO_2 to CO or formate with Co^{II}, Ru^{II}, and Re^{II} complexes. Photoreduction of CO_2 to organic products has been achieved in low yields by use of semiconductor powders[16,17] such as strontium titanate ($SrTiO_3$), tungsten oxide (WO_3), and titanium oxide (TiO_2).

Recently[18] we have demonstrated that carbon dioxide is photoreduced to methane by heterogeneous Ru or Os colloids. The reductive insertion of CO_2 into a carbon–hydrogen bond [equation (1)] might be considered as a simple route for the fixation of CO_2. In nature many enzymes are active in decarboxylation processes that are anti-CO_2-fixation routes. For example, in the catabolic cycle, isocitric and malic acids are decarboxylated to α-oxoglutaric acid [equation (2)] and pyruvic acid [equation (3)] in the presence of the cofactor nicotinamide adenine dinucleotide phosphate ($NADP^+$) and the appropriate enzyme isocitrate dehydrogenase (ICDH) (E.C. 1.1.1.42) or malic enzyme (E.C. 1.1.1.40). Similarly, formate is decarboxylated in the presence of the cofactor nicotinamide adenine dinucleotide (NAD^+) and catalysed by formate dehydrogenase (FDH) (E.C. 1.2.1.2) [equation (4)].

Since enzymes are often reversibly active, one might envisage means to reverse the processes occurring in nature and induce carboxylation pathways using biocatalysts, and thereby develop CO_2-fixation processes. Recently[19,20] we have



(1) (2) (3) (4)

developed photoinduced cycles for the regeneration of the NAD(P)H cofactors. We have shown that the photogenerated *N,N'*-dimethyl-4,4'-bipyridinium radical $MV^{+\bullet}$ mediates the reduction of NAD^+ or $NADP^+$ to NADH and NADPH in the presence of the enzymes lipoamide dehydrogenase (LipDH) (E.C. 1.6.4.3) and ferredoxin-$NADP^+$-reductase (FDR) (E.C. 1.18.1.2), respectively. Furthermore, we were able to couple the photoinduced regeneration cycles to enzyme-catalysed synthesis of amino acids, alcohols, and hydroxy acids. Thus, it seems feasible that CO_2 reduction processes might be driven by the light-induced regeneration of NAD(P)H cofactors, assuming that the enzymes exhibit reversible activity.

In a preliminary note, we have reported[1] on the successful photoinduced carboxylation of pyruvic acid to form malic acid as well as the fixation of CO_2 into α-oxoglutaric acid to form isocitric acid. Here we present a comprehensive study of several photoinduced enzyme-catalysed CO_2 fixation systems.

These fixation processes were carried out by using either the NAD(P)H photoregeneration cycles coupled to enzymic fixation reactions, or by utilizing an artificially reduced cofactor coupled to an enzyme.

Specifically, we have developed artificial photochemical systems for the reductive carboxylation of pyruvic and α-oxoglutaric acids to malic and isocitric acids, respectively. Also the reduction of CO_2 to formate has been accomplished by use of various relay systems and formate dehydrogenase as biocatalyst.

Malic acid obtained by the fixation of CO_2 into pyruvic acid could be utilized as a synthetic precursor for sesquestered enzyme-catalysed reaction. Specifically, this photoproduct has been coupled to dehydration followed by amination to form the C_4-aspartic acid product.

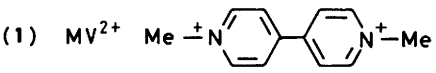
Experimental

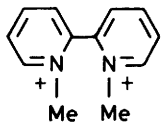
Absorption spectra were recorded with a Uvikon-820 (Kontron) spectrophotometer. Product analyses were performed with an LKB high-pressure liquid chromatograph equipped with Knauer UV and conductivity detectors or with an LKB 4400 amino acid analyser.

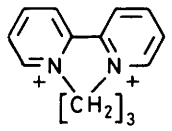
Illumination experiments were performed in 5 ml glass cuvettes (1 × 1 cm) equipped with a small stirring bar and a serum stopper. The light source was a 1 000 W halogen quartz lamp, and light was filtered through a 400 nm cut-off filter. Comparison of the different relay systems in formate formation was carried out by placing all the couvettes on a rotating disc. The disc was immersed in a water-bath, kept at 10 °C, and rotated by an external motor. *N,N'*-Dimethyl-2,2'-bipyridinium (DM^{2+}) (**2**), *N,N*-trimethylene-2,2'-bipyridinium (DT^{2+}) (**3**), and *N,N'*-tetramethylene-2,2'-bipyridinium (DQ^{2+}) (**4**) were prepared according to the literature methods.[21] All other materials and enzymes were purchased from Sigma or Aldrich.

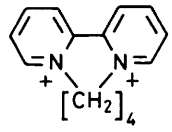
Malic acid. The system was composed of a Tris buffer (4.2 ml; 0.2M; pH 8.0) that included the substrate: pyruvic acid (4.7×10^{-2}M), MV^{2+} (1.9×10^{-4}M), $NADP^+$ (1.8×10^{-4}M), $[Ru(bpy)_3]^{2+}$ (2.1×10^{-5}M), $MnCl_2$ (9.5×10^{-5}M), $NaHCO_3$ (0.2M), and the electron donor 2-mercaptoethanol (1.9×10^{-2}M initial concentration). To this solution were added FDR (0.2 units) and malic enzyme (1.33 units). The system was illuminated under a gaseous atmosphere of CO_2 and the pH was maintained at 7.9 by gradual addition of KOH. Samples (300 μl) of the solution were taken out at intervals, passed through a cation-exchange resin (Dowex 50W × 8), and analysed by h.p.l.c. coupled with a Wescan anion, exclusion column and a conductivity detector (eluant solution 10^{-3}M-H_2SO_4; flow 0.6 ml min^{-1}).

Aspartic acid. The system was composed of an aqueous 0.16M-Tris buffer solution (4 ml; pH 7.9) that included $[Ru(bpy)_3]^{2+}$ (2.3×10^{-5}M), $NaHCO_3$ (0.16M), NH_4^+ (0.08M), pyruvic acid (4×10^{-2}M), MV^{2+} (1.6×10^{-4}M), $NADP^+$ (3.2×10^{-4}M), $MnCl_2$ (8×10^{-5}M), and 2-mercaptoethanol (1.6×10^{-2}M initial concentration). To the solution were added the enzymes FDR (0.5 units), malic enzyme (0.5 units), fumarase (E.C. 4.2.1.2; 156 units) and L-aspartase (E.C. 4.3.1.1; 3 units). The deaerated solution was illuminated and samples (100 μl) were taken out at intervals. Samples were treated with 5-sulphosalicylic[22] acid before analysis. Malic acid and fumaric acid were analysed by ion chromatography using the procedure described for malic acid. Aspartic acid was analysed by an amino acid analyser.

(1) MV^{2+} 

(2) DM^{2+} 

(3) DT^{2+} 

(4) DQ^{2+} 

Isocitric acid. The system was composed of Tris buffer (4.2 ml; 0.2M; pH 7.2) that included $[Ru(bpy)_3]^{2+}$ (1.4×10^{-5}M), MV^{2+} (1.7×10^{-4}M), $NADP^+$ (1.7×10^{-4}M), $MnCl_2$ (1.7×10^{-3}M), $NaHCO_3$ (0.17M), α-oxoglutaric acid (4.2×10^{-2}M), and the electron donor DL-dithiothreitol (DTT) (8.3×10^{-3}M initial concentration). The following enzymes were added: FDR (0.2 units) and isocitrate dehydrogenase (0.47 units) immobilized on poly(acrylamide-co-*N*-acryloxysuccinimide) by the procedure developed by Whitesides *et al.*[23] Samples (120 μl) of the illuminated solution were passed through a cation-exchange resin (Dowex 50W × 8) and analysed on an RP-18 column (Merck); eluant 0.3M-H_3PO_4 (0.8 ml min^{-1} flow rate). Isocitric and α-oxoglutaric acids were detected at 210 nm.

Formic acid. To a phosphate buffer (3.4 ml; 0.2M; pH 7.0) solution were added $[Ru(bpy)_3]^{2+}$ (3×10^{-5}M), one of the charge relays (MV^{2+}, DM^{2+}, DT^{2+} or DQ^{2+}; 1×10^{-3}M), cysteine as an electron donor (1×10^{-2}M), $NaHCO_3$ (0.2M), and formate dehydrogenase (FDH) (0.89 units). Samples (200 μl) were taken out at intervals and analysed for formate using an ion chromatograph (Wescan anion-exclusion column; eluant 10^{-3}M-H_2SO_4; flow rate 0.6 ml min^{-1}), as well as by an enzymic assay.[24]

Results and Discussion

Illumination under CO_2 of the aqueous solution that includes the photosystem $[Ru(bpy)_3]^{2+}$ as sensitizer, the charge relay MV^{2+}, and the sacrificial electron donor 2-mercaptoethanol, in the presence of the cofactor $NADP^+$, the substrate pyruvic acid, and the enzymes FDR and malic enzyme, results in the formation of malic acid. The rate of product formation is depicted in Figure 1. Control experiments revealed that all the

components are essential for the photoinduced production of malic acid, and exclusion of any of the components of the system prevented the formation of malic acid.

Our previous studies[19] have elucidated the different steps involved in the photoregeneration of NADPH. The excited sensitizer $[Ru(bpy)_3]^{2+}$* is quenched by MV^{2+} *via* an electron-transfer process. The oxidized sensitizer formed by this electron-transfer reaction is reduced and consequently the light-active compound is regenerated. The reduced electron relay $MV^{+\bullet}$ mediates the reduction of $NADP^+$ in the presence of the biocatalyst FDR. Indeed, upon illumination of a solution which contains $[Ru(bpy)_3]^{2+}$, MV^{2+}, 2-mercaptoethanol, $NADP^+$, and the enzyme FDR, accumulation of NADPH could be monitored spectroscopically at λ 340 nm (φ = 1.9%). Addition of the malic enzyme in the dark, in the presence of the substrate, pyruvic acid, and CO_2, resulted in the disappearance of NADPH and the production of malic acid. Thus, we conclude that the photoinduced reduction of $NADP^+$ mediates the reductive carboxylation of pyruvic to malic acid.

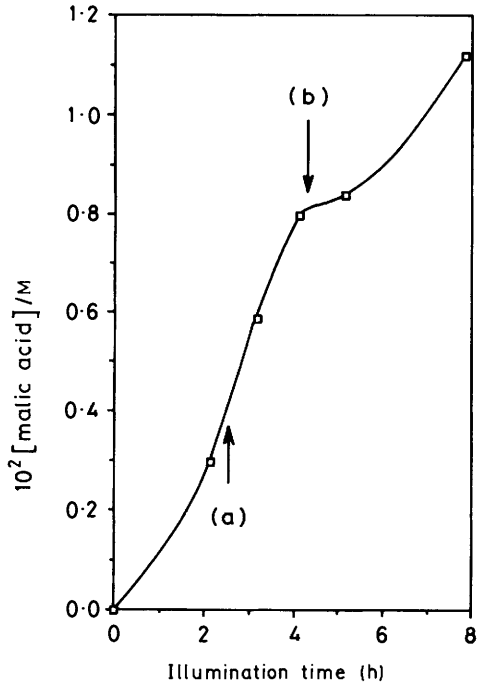
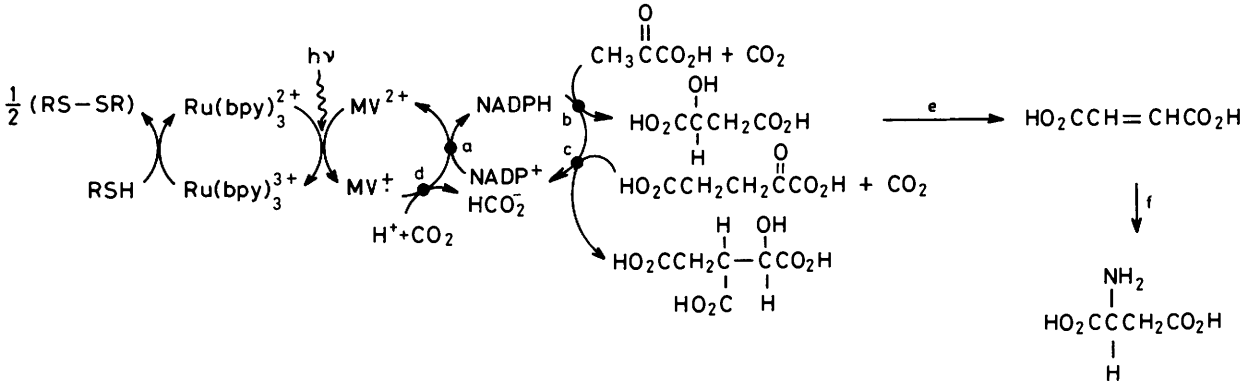


Figure 1. Rate of malic acid formation as a function of illumination time with addition of mercaptoethanol at (a) (2.2×10^{-2}M) and (b) (2.7×10^{-2}M)

Figure 2 summarizes schematically the sequence of catalytic reactions that leads to the fixation of CO_2 into pyruvic acid. Several points should be noted. The nature of the electron donor strongly affects the fixation process. Substitution of 2-mercaptoethanol by other electron donors such as ethylenediaminetetra-acetic acid (EDTA), triethanolamine, or cysteine totally inhibited the formation of malic acid. Since our previous studies have indicated that these electron donors lead to photoinduced regeneration of NADPH, we conclude that the latter donors deactivate the carboxylation catalyst, namely the malic enzyme. In addition, the rate of NADPH formation must be controlled, since accumulation of NADPH in continuous illumination results in degradation of the cofactor. The lability of the cofactor is probably due to partial photochemical activity[25] of the reduced cofactor NADPH that might serve as an electron donor to the oxidized sensitizer. Hence, we had to monitor the rate of NADPH formation, so that a low steady-state concentration of the reduced cofactor was maintained. The experimental conditions described represent a suitable balance for the photochemical generation of NADPH and subsequent chemical consumption of the cofactor. Thus the quantum yield of carboxylated acids is controlled by the quantum yield of NADPH formation,[19] φ = 1.9%. Indeed, it can be shown (Figure 1) that the rate of malic acid formation is maintained constant over a long period of illumination.

Our success in the photocarboxylation of pyruvic acid to form malic acid has encouraged us to try to use photosynthesized malic acid as a 'building block' for the photoinduced synthesis of other C_4-products by enzymic means. Malic acid can, in principle, be dehydrated enzymically to form fumaric acid, that subsequently, in the presence of another enzyme (aspartase) and ammonium ions, is aminated to aspartic acid.

Illumination of an aqueous system that includes all the components described for the photoinduced production of malic acid, and in addition ammonium ions and the enzymes fumarase and aspartase, yields aspartic acid. Figure 3 displays the rate of aspartic acid formation at intervals of illumination. In the absence of aspartase no aspartic acid is formed and the photoproducts are malic acid and fumaric acid. However in the presence of aspartase only aspartic acid is detected as photoproduct and no accumulation of the intermediate products, *i.e.* malic or fumaric acid, can be detected in the reaction medium. These results are attributed to the equilibrium constant of the last reaction in the sesquestered enzymic cycle, *i.e.* the amination process ($K = 4.3 \times 10$ l mol^{-1}). While the conversion of malic into fumaric acid is unfavourable (K =



a, ferredoxin–$NADP^+$–reductase; b, malic enzyme; c, isocitrate dehydrogenase; d, formate dehydrogenase; e, fumarase; f, aspartase

Figure 2. Cyclic scheme for the photoinduced CO_2-fixation systems

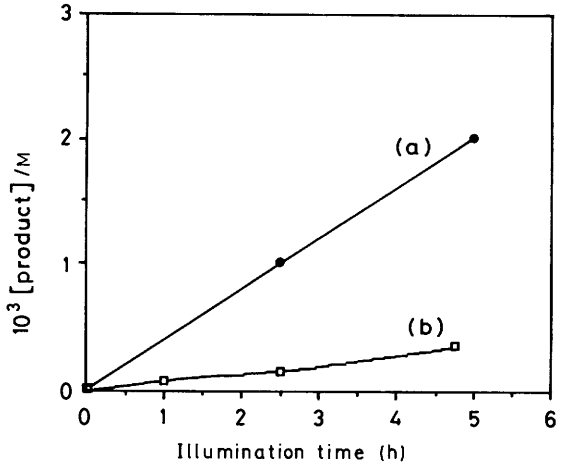


Figure 3. Rate of (a) aspartic and (b) fumaric acid formation as a function of illumination time

0.23), coupling of this reaction to the formation of aspartic acid shifts the equilibrium towards amino acid formation, and consequently, in the presence of aspartase, aspartic acid is the only product.

Figure 2 represents schematically the entire photochemical process leading to the fixation of CO_2 into pyruvic acid to form aspartic acid *via* a multi-enzyme-catalysed process.

A similar approach has been examined for the fixation of CO_2 into α-oxoglutaric acid to produce isocitric acid. Illumination of an aqueous solution under CO_2 that includes $[Ru(bpy)_3]^{2+}$, the relay MV^{2+}, the electron donor DTT, $NADP^+$, the substrate α-oxoglutaric acid, and the two enzymes FDR and isocitrate dehydrogenase (ICDH) yields only low amounts of isocitric acid (*ca.* 10^{-4}M), and the system is deactivated after 1 h illumination. Re-addition of ICDH regenerates the activity of the system towards isocitric acid formation upon illumination, implying that the carboxylating enzyme is deactivated in the artifical environment. We therefore immobilized the ICDH on a water-soluble polyacrylamide derivative containing active ester groups.[23]

Illumination of the previously described system with the immobilized ICDH resulted in the long-term formation of isocitric acid. Figure 4 displays the rate of product formation as a function of illumination time. It is evident that the activity of the system is maintained constant for at least 6 h illumination. Control experiments revealed that all the components included in the system are essential to induce the photosynthesis of isocitric acid, and exclusion of any one component prevents product formation. A stepwise experiment, where ICDH was added in the dark to an aqueous solution that included photogenerated NADPH, was also performed. Addition of the second enzyme (ICDH) resulted in the disappearance of NADPH and formation of isocitric acid. Thus, it is evident that the production of isocitric acid proceeds by a mechanistic cycle similar to that described for malic acid formation (Figure 2). The primary step involves the FDR-catalysed photosensitized regeneration of NADPH; the second step utilizes the reduced cofactor to induce the enzyme-catalysed carboxylation of α-oxoglutaric to isocitric acid [equation (2)].

The enzymic decarboxylation of formic acid by NAD^+ [equation (4)] in the presence of formate dehydrogenase is well established.[26] This process is thermodynamically favoured towards the oxidation of formic acid ($\Delta G° = -4.6$ kcal mol^{-1}).*

Thus, better reducing agents than NADH must be used in order to reverse the naturally occurring process. Previous studies[27] revealed that FDH (from *Pseudomonas oxalaticus*) recognizes various artifical electron relays in addition to NAD^+,

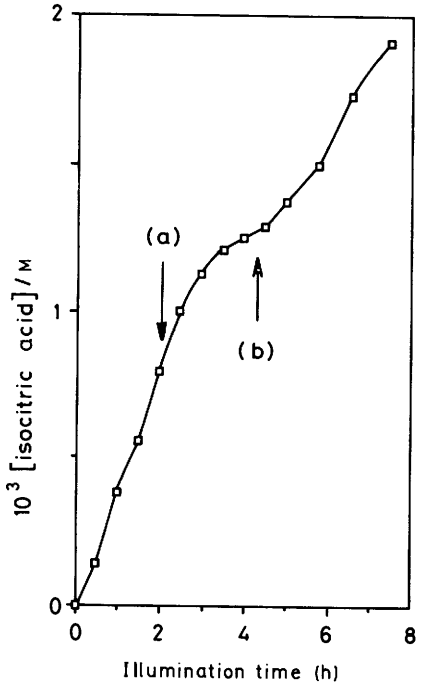


Figure 4. Rate of isocitric acid formation as a function of illumination time with addition of DTT (5×10^{-3}M) at (a) and (b)

such as methylviologen and benzylviologen radical cations. Therefore, we have examined the ability of a series of reduced relays of bipyridinium structure to act as artificial cofactors for the reduction of CO_2 in the presence of the enzyme FDH.

Interestingly, we have found that several reduced bipyridinium species act as substrates for the enzyme FDH, and the participation of the cofactor, NAD^+, is not required. Illumination of aqueous solutions under CO_2 (pH = 6.8) that include the sensitizer $[Ru(bpy)_3]^{2+}$, one of the electron relays *N,N'*-dimethyl-4,4'-bipyridinium (MV^{2+}) (**1**), *N,N'*-dimethyl-2,2'-bipyridinium (DM^{2+}) (**2**), *N,N'*-trimethylene-2,2'-bipyridinium (DT^{2+}) (**3**), and *N,N'*-tetramethylene-2,2'-bipyridinium (DQ^{2+}) (**4**), cysteine as electron donor, and the enzyme formate dehydrogenase, results in the reduction of CO_2 to formate. The rate of formate formation at intervals of illumination is displayed in Figure 5. Control experiments revealed that all the components are essential to induce the reduction of CO_2, although very small amounts of formate could be detected in the absence of FDH. It should be emphasized that the enzyme FDH is very unstable; upon illumination of these aqueous systems at room temperature (24 °C) complete deactivation of the enzyme is observed within 0.5 h and only limited amounts of formate can be accumulated.

Attempts to stabilize the enzyme through immobilization failed. Nevertheless, by lowering the temperature of the reaction medium the stability of the enzyme is substantially enhanced. Illumination of the aqueous solutions was therefore performed at 10 °C. Under these conditions, the concentrations of formate produced were in the 10^{-3}M region (Figure 5). However, even under these conditions deactivation of FDH occurs: the enzyme loses 90% of its initial activity within 6 h illumination. The highest quantum yields for formate formation are observed using the two relays MV^{2+} and DT^{2+} and correspond to $\varphi = 1.6 \times 10^{-2}$.

The fact that the various reduced relays mediate directly the reduction of CO_2 to formate in the presence of FDH allows us

* 1 kcal = 4.184 kJ.

to outline the cyclic scheme displayed in Figure 2 as the route for the formation of formate.

It should be noted that the effectiveness of the various relay systems for the reduction of CO_2 to formate does not coincide with their reduction potentials. The quantum yield for the formation of the reduced relay is affected by the quenching rate [equation (5)] and the destructive recombination of the photoproducts by back electron-transfer [equation (6)].

$$[Ru(bpy)_3]^{2+}* + V^{2+} \xrightarrow{k_a} [Ru(bpy)_3]^{3+} + V^{+\bullet} \quad (5)$$

$$[Ru(bpy)_3]^{3+} + V^{+\bullet} \xrightarrow{k_b} [Ru(bpy)_3]^{2+} + V^{2+} \quad (6)$$

Previous studies[28] have indicated that the effectiveness of electron transfer from excited $[Ru(bpy)_3]^{2+}$ to a series of bipyridinium charge relays depends strongly upon the reduction potential of the relay. It has been found that the quenching rate constant decreases as the reduction potential of the relay decreases, consistent with electron-transfer theories. In contrast, the back electron-transfer process is only slightly affected by the reduction potential, and with all relay systems used in our studies diffusion-controlled rate constants have been observed. Thus, the effectiveness of the reduced relay production is affected mainly by the electron-transfer quenching process.

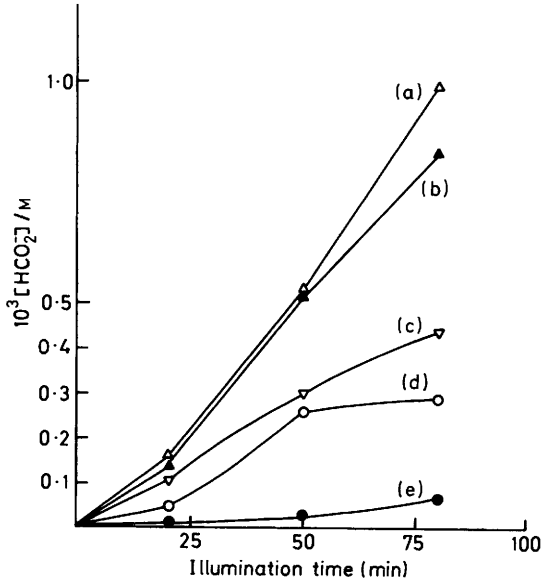


Figure 5. Rate of formate formation as a function of illumination time using various charge relays: (a) MV^{2+}, (b) DT^{2+}, (c) DQ^{2+}, (d) DM^{2+}, (e) DT^{2+} without FDH

Table 1. Quantum yields, reduction potentials, and quenching constants of the charge relays in the formic acid system

Electron relay	$E°$ (28 °C)/ V *vs.* NHE	10^{-8} k_q (28 °C)/ l mol^{-1} s^{-1}	φ (%)
MV^{2+}	−0.44	10.3	1.6
DT^{2+}	−0.55	5.74	1.6
DQ^{2+}	−0.65	3.24	1.0
DM^{2+}	−0.72	1.8	0.5

Table 1 summarizes the quantum yields for formic acid formation by use of the various relays in comparison with their reduction potentials and electron-transfer quenching rate constants. It is evident that the highest formate yields are obtained with MV^{2+}, which exhibits superior quenching properties, while DM^{2+} shows the lowest activity as well as a poor electron-transfer quenching rate constant. Thus, it is reasonable to attribute the different quantum yields of formic acid production with the various relay systems to their primary electron-transfer quenching properties. Nevertheless, differences in recognition of the reduced relay by the enzyme FDH and subsequent effects on formic acid production cannot be excluded.

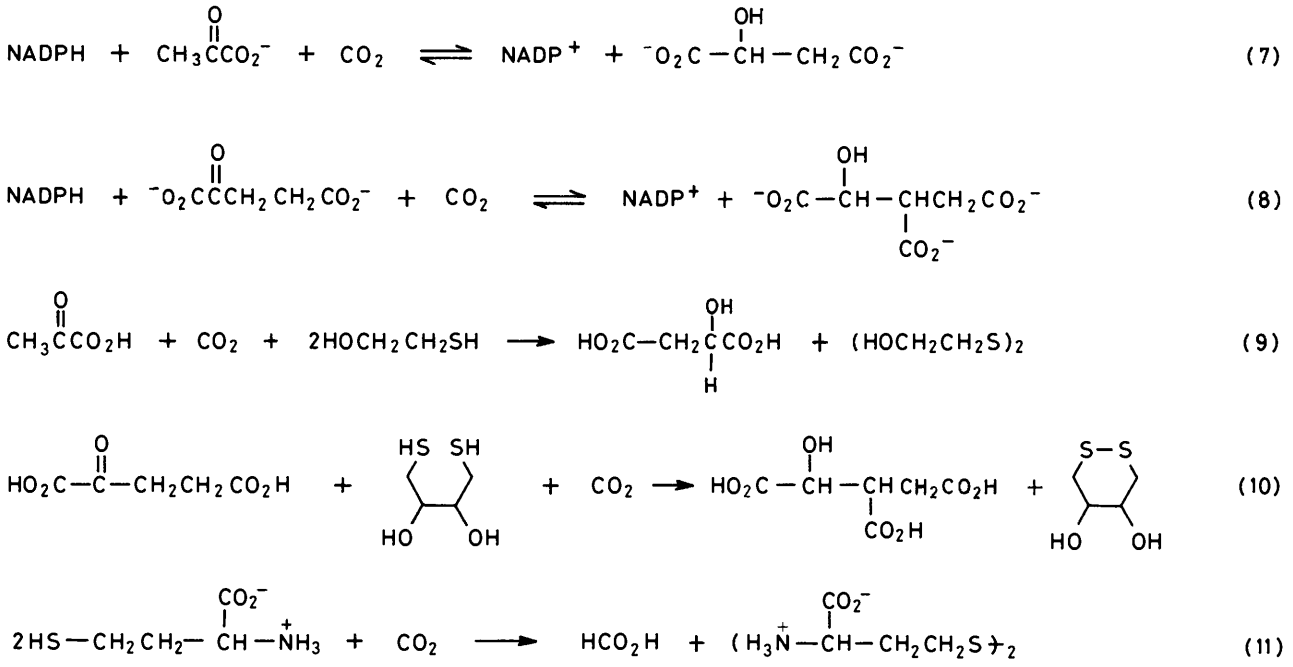
Whenever enzymes are applied as biocatalysts in synthesis, and specifically when they are introduced into artifically tailored systems, their stability must be considered. Table 2 summarizes the turnover numbers (TN) of the enzymes as well as other ingredients included in the various CO_2-fixation routes. The quoted turnover numbers for the enzymes are lower limits in the sense that enzyme activity is still preserved when these values are determined. It is evident that the enzymes as well as other ingredients are effectively recycled in the various systems. However, we emphasize that different precautions were undertaken to achieve the observed stabilities of the biocatalysts. For malic enzyme, selection of the specific electron donor, *i.e.* 2-mercaptoethanol, and controlling the rate of NADPH photogeneration are essential to maintain the activity of the system. For ICDH, immobilization on a solid support is required to achieve biocatalyst stabilization. Finally, stabilization of FDH is accomplished by operating the photoinduced CO_2-fixation process at relatively low temperatures (10 °C).

A further aspect to be considered in these systems relates to the thermodynamic balance of the net photosensitized CO_2-fixation processes. We have emphasized earlier[29] the synthetic advantages of using the photochemically induced regeneration of cofactors over chemical regeneration routes. The fact that light energy constantly drives the reduction of $NAD(P)^+$ to NAD(P)H through the photosensitized process eliminates the reverse reduction of $NAD(P)^+$ by the product as it is accumulated. Consequently, endoergic reactions can be derived and products can be accumulated in amounts that are substantially higher than the estimated dark-equilibrium values. The carboxylation processes of pyruvic acid and α-oxoglutaric acid by NADPH to form malic and isocitric acids, respectively

Table 2. Turnover numbers of the components in the various CO_2-fixation reactions.

	$[Ru(bpy)_3]^{2+}$	MV^{2+}	$NADP^+$	FDR[a]	FDH[b]	Malic enzyme[c]	ICDH[d]	Fumarase[e]	Aspartase[f]
Malic acid	1 074	117	62.2	2.3×10^4		7.4×10^5			
Aspartic acid	174	25	6.3	1.6×10^3		6.3×10^4		8.5×10^2	5.2×10^2
Isocitric acid	272	23	11.4	2.5×10^3			5.5×10^4		
Formic acid	67	2			2×10^3				

[a] Formula wt. (FW) 40 000 (M. Shin, *Methods Enzymol.*, 1971, **23**, 441). [b] FW 300 000 (T. Hopner and A. Trutwein, *Z. Naturforsch., Teil B*, 1972, **27**, 1075). [c] FW 280 000 (R. Y. Hsu and H. A. Lardy, *J. Biol. Chem.*, 1967, **242**, 520). [d] FW 58 000 (R. F. Colman, *J. Biol. Chem.*, 1968, **243**, 2454). [e] FW 48 500 (S. Beekmans and L. Kanarek, *Eur. J. Biochem.*, 1977, **78**, 437). [f] FW 48 500 (S. Suzuki, J. Yamaguchi, and M. Tokushige, *Biochim. Biophys. Acta*, 1973, **321**, 369).



[equations (7) and (8)] exhibit equilibrium constants of 19.6 and 1.3 l mol^{-1}, respectively. Thus, after light-induced generation of NADPH, the two carboxylation reactions are expected to proceed spontaneously.

Nevertheless, the net photosensitized CO_2-fixation processes that form malic and isocitric acids (Figure 2) correspond to the reductive carboxylation of pyruvic and α-oxoglutaric acids by 2-mercaptoethanol [equation (9)] and D,L-dithiothreitol (DTT) [equation (10)], respectively. The CO_2-fixation process to form malic acid is endoergic by *ca.* 11.5 kcal mol^{-1} of product formed; in turn, the thermodynamic balance for the process that forms isocitric acid is estimated to be close to $\Delta G° \simeq 0$. Since DTT is a relatively powerful reducing agent ($E°$ *ca.* −0.3 V *vs.* hydrogen electrode), the process might exhibit endoergic properties if this electron donor was substituted with donors of weaker reducing properties, *i.e.* expected to lead to endoergic production of isocitric acid.

Finally, the fixation of CO_2 to formic acid by using FDH corresponds to the light-induced reduction of CO_2 by cysteine [equation (11)]. The thermodynamic balance of this process shows that it is endoergic by *ca.* 5.5 kcal. mol^{-1} of formate formed and demonstrates that in this process light energy is converted and stored in the form of formic acid that might be considered as a fuel.

Conclusions

The present study has revealed two approaches to the fixation of CO_2 where enzymes and natural cofactors act as catalysts in artificial chemical environments. One approach involves the carboxylation of α-oxo acids by CO_2 and elongation of the organic substrate chain by a single carbon atom. These carboxylation reactions represent the reverse of some processes that occur in the catabolic cycle in nature. For example, the light-induced formation of isocitric acid corresponds to the reverse of one step of the Krebs cycle where decarboxylation occurs. The photoinduced carboxylation reaction allows us to form synthetic building blocks for subsequent sesquestered enzymic synthesis. The formation of aspartic acid from pyruvic acid demonstrates a route for the derivatization of organic substrate through the primary fixation of CO_2. A second approach for the fixation of CO_2 is exemplified by the formation of formate in the absence of a natural cofactor but with the enzyme FDH as biocatalyst. We have shown that various reduced relay systems act as artificial cofactors for the enzyme. Formate as product is of substantial interest as it acts as a hydrogen storage compound. Subsequent H_2 evolution or utilization of the formate hydride in hydrogenation reactions might be important routes.

The application of biocatalysts in artificial media reveals some complexity in tailoring the systems. Stabilization of the biocatalysts has been accomplished *via* specific and different methods such as immobilization on polymers, inclusion of electron donors that stabilize the enzymes, or lowering the temperature of the reaction medium.

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