Photoinduced Carbon Dioxide Fixation forming Malic and Isocitric Acid

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Photoinduced CO_2-fixation into organic substrates is accomplished *via* enzyme-catalysed reactions.

Carbon dioxide fixation into the form of organic compounds is a major challenge of chemistry. Of particular interest is visible-light induced CO_2 fixation as a means of mimicking natural photosynthesis. Photosensitized NADPH regeneration cycles can be utilized in CO_2 fixation to form malic and isocitric acid. Photosensitized CO_2-fixation into organic compounds such as formic and oxalic acid has been reported recently. In these reports semiconductors[1—4] or homogeneous catalysts[5,6] mediate CO_2 reduction. The specificity and efficiency of these processes is limited. The photoinduced fixation of CO_2 to formic acid has also been reported using a semiconductor–enzyme catalysed system.[7] We have recently reported[8] on the enzyme catalysed photosensitized regeneration of NADPH. In this system photogenerated methylviologen radical, $MV^{\cdot+}$, mediates the reduction of $NADP^+$ to NADPH in the presence of ferredoxin–$NADP^+$ reductase (E.C. 1.18.1.2).

Here we report successful CO_2 fixation forming malic acid (**1**) and isocitric acid (**2**), using NADPH dependent enzymes coupled to the photosensitized NADPH regeneration system. The system for malic acid formation is composed of an aqueous solution (4.2 ml; Tris buffer, 0.2 M; pH 8.0) that includes trisbipyridineruthenium, $Ru(bpy)_3^{2+}$ (2.1×10^{-5} M) as sensitizer, methyl viologen, MV^{2+} (1.9×10^{-4} M), as primary electron acceptor, $NADP^+$ (1.8×10^{-4} M), $MnCl_2$ (9.5×10^{-5} M), $NaHCO_3$ (0.2 M), pyruvic acid (4.7×10^{-2} M), and the electron donor mercaptoethanol (1.9×10^{-2} M, initial concentration). Two enzymes are included in the system: ferredoxin–$NADP^+$ reductase (FDR, E.C. 1.18.1.2, 0.2 Units) and the malic enzyme (E.C. 1.1.1.40, 1.33 Units). Illumination of this system ($\lambda > 400$ nm) under a CO_2 atmosphere (1.01 atm) results in the formation of malic acid (**1**).

The rate of malic acid formation as a function of illumination time was followed by h.p.l.c. [Figure 1(A)]. In the absence of malic enzyme no malic acid was formed and NADPH accumulated in the system as a photoproduct. Introduction of the malic enzyme resulted in the disappearance of NADPH and formation of (**1**).

In view of our previous observations,[8] we suggest the schematic cycle outlined in Figure 2 as the multi-step process leading to the formation of malic acid. The primary photochemical process involves the photosensitized reduction of MV^{2+} by the electron donor using $Ru(bpy)_3^{2+}$ as sensitizer. The characterization of the photosensitized reduction of viologens by various sensitizers such as $Ru(bpy)_3^{2+}$ or Zn-porphyrins has been established in numerous studies.[9,10] The subsequent reaction involves the enzyme catalysed reduction of $NADP^+$ by $MV^{\cdot+}$ to form NADPH. $NADP^+$ can be reduced by two alternative routes:[11] a single-electron-transfer reduction (E° – 0.85 V *vs.* normal hydrogen electrode, N.H.E.) that leads to $NAD^\cdot$ which can undergo dimerization to a biologically inactive reduction product,[11,12] or hydride reduction to form the biologically active NADPH

Table 1. Turnover numbers of components involved in the CO_2 fixation processes.

	$Ru(bpy)_3^{2+}$	MV^{2+}	$NADP^+$	FDR[a]	Malic enzyme[b]	ICDH[c]	% Conversion
Malic acid	1074	117	62.2	2.3×10^4	7.4×10^5		24
Isocitric acid	272	23	11.4	2.5×10^3		5.5×10^4	4.6

[a] $M_r \sim 40000$; *cf.*: M. Shin, *Methods Enzymol.*, 1971, **23**, 441. [b] $M_r \sim 280000$; *cf.*: R. Y. Hsu and H. A. Lardy, *J. Biol. Chem.*, 1967, **242**, 520. [c] $M_r \sim 58000$; *cf.*: R. F. Colman, *J. Biol. Chem.*, 1968, **243**, 2454.

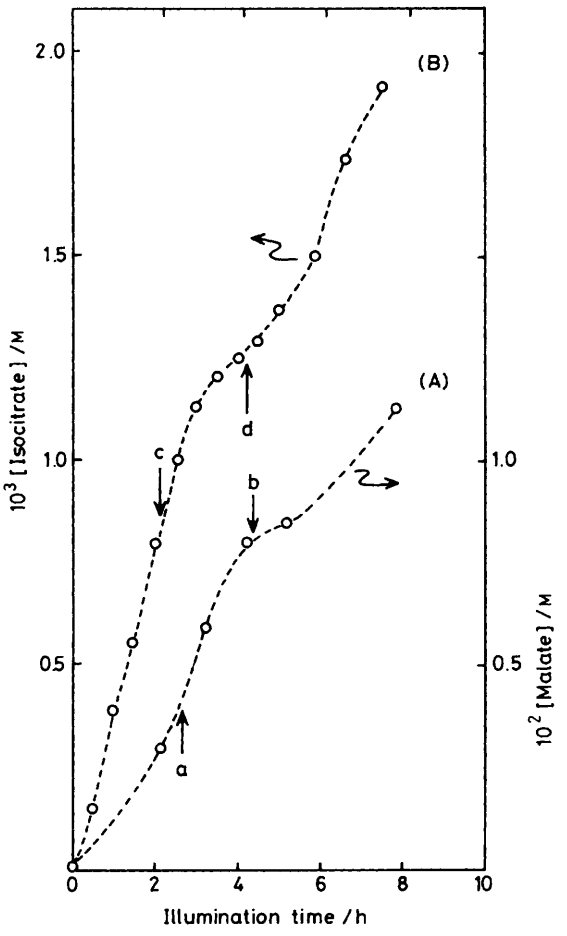


Figure 1. Rate of CO_2 fixation as a function of illumination time. (A) Pyruvic acid: a, addition of mercaptoethanol (2.2×10^{-2} M); b, addition of mercaptoethanol (2.7×10^{-2} M); (B) oxoglutaric acid: c and d addition of DTT (5×10^{-3} M).

(E° − 0.32 V *vs.* N.H.E.). Previous studies have indicated that hydride compounds such as $NaBH_4$[11] or hydrido-Rh complexes[13,14] are capable of reducing $NADP^+$ to NADPH *via* the second route. Other reports have shown[15,16] that the enzyme FDR catalyses the reduction of $NADP^+$ by $MV^{\bullet+}$. Since $MV^{\bullet+}$ is thermodynamically unable to reduce $NADP^+$ to its radical (E° − 0.44 V *vs.* N.H.E.), we presume that the process is accomplished *via* a hydride intermediate formed at the active site of the enzyme. The photoproduced NADPH mediates the fixation of CO_2 into pyruvic acid forming malic acid in the presence of the malic enzyme, and $NADP^+$ is recycled for the photochemical reaction.

The net reaction accomplished in this system corresponds to CO_2 fixation into pyruvic acid using mercaptoethanol as electron donor (equation 1). The thermodynamic balance of this reaction shows that it is endoenergic by *ca.* 11.5 kcal per mole of mercaptoethanol consumed (1 cal = 4.184 J).

The system for the photogeneration of isocitric acid (**2**) is composed of an aqueous solution (4.2 ml; Tris buffer, 0.2 M; pH 7.2) that includes $Ru(bpy)_3^{2+}$ (1.4×10^{-5} M), $NaHCO_3$ (0.17 M), oxoglutaric acid (4.15×10^{-2} M), D,L-dithiothreitol (DTT, 8.3×10^{-3} M initial concentration) as electron donor, and the two enzymes ferredoxin–$NADP^+$ reductase (FDR, E.C. 1.18.1.2, 0.2 Units) and isocitrate dehydrogenase (ICDH, E.C. 1.1.1.42, 0.47 Units immobilized on PAN[17]).

Illumination of this system ($\lambda > 420$ nm) under an atmosphere of CO_2 (1.08 atm) results in the fixation of CO_2 into oxoglutaric acid and formation of isocitric acid (**2**). The rate of isocitrate formation as a function of illumination time is shown in Figure 1(B).

Control experiments reveal that all components are required in the system. The sequence of reactions that lead to the formation of isocitric acid are summarized in Figure 2 and involve the utilization of photogenerated NADPH in the fixation of CO_2 into oxoglutaric acid in the presence of isocitrate dehydrogenase. The net reaction forming (**2**) corresponds to the light-induced fixation of CO_2 into oxoglutaric acid using D,L-dithiothreitol (DTT) as electron donor (equation 2). The thermodynamic balance of this process is approximately zero. [E° (DTT) −0.3 V *vs.* N.H.E.]

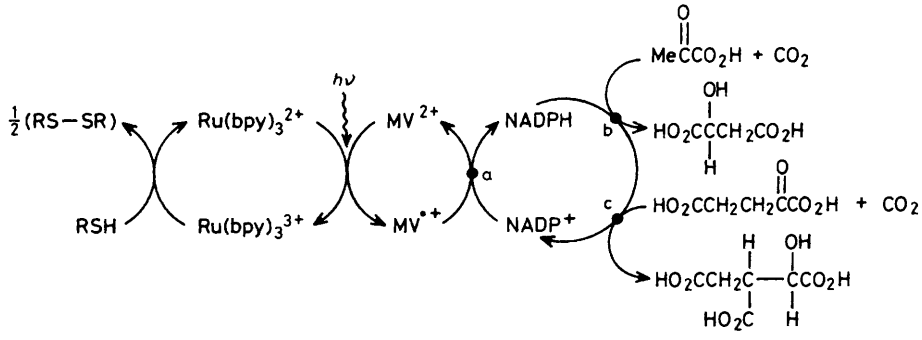
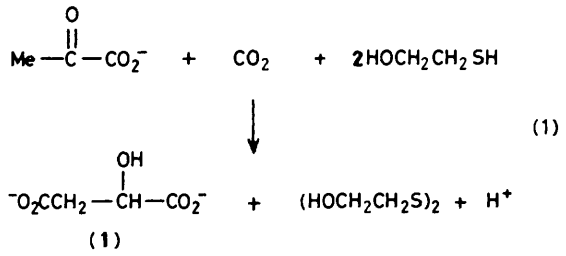
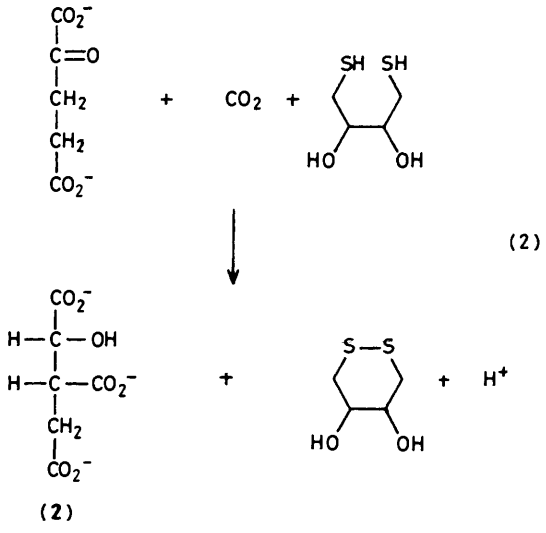


Figure 2. Cyclic scheme for the photoinduced regeneration of NADPH and CO_2 fixation into keto acids. a, Ferredoxin–$NADP^+$ reductase; b, malic enzyme; c, isocitrate dehydrogenase.



(1)



(2)

The stability of the different components involved in the formation of (**1**) and (**2**) was examined and the turnover numbers of the different components are summarized in Table 1. These data correspond to 24% conversion of pyruvic acid into (**1**) and 4.6% conversion of oxoglutaric acid into (**2**).

It should be noted that in the two processes the efficiency of NADPH production must be tuned to a low level since the CO_2 fixation reactions are the rate determining steps. Accumulation of NADPH results in substantial decomposition of this ingredient.

In conclusion, we have accomplished the enzyme catalysed fixation of CO_2 into organic substrates using the photosensitized NADPH regeneration cycle. One of these processes is endoenergic and represents a process that converts light energy into chemical potential.

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