Photoinduced Enzyme-catalysed Synthesis of Amino Acids by Visible Light

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Visible light-induced NADPH regeneration effects the production of glutamic acid that mediates transamination and formation of aspartic acid and alanine in the presence of enzymes.

Application of light-induced electron-transfer reactions in the development of photocatalysed and photosynthetic processes is of general interest.[1,2] Also of interest is the regeneration of cofactors that might be coupled to enzyme-catalysed synthesis. Chemical[3] and electrochemical[4] regeneration of cofactors has been widely described. The photosensitized regeneration of 1,4-dihydronicotinamide adenine dinucleotide phosphate (NADPH), using visible light, has recently been described by us.[5,6] In this system photogenerated *N*,*N*′-dimethyl 4,4′-bipyridinium radical (methylviologen, $MV^{\bullet+}$) mediates

Table 1. Turnover numbers of the components in the different enzyme-catalysed reactions.

	$Ru(bpy)_3^{2+}$	MV^{2+}	$NADP^+$	FDR[a]	GluDH[b]	GOT[c] or GPT[d]
Glutamic acid	1220	31	16	2.6×10^4	7.4×10^6	
Aspartic acid	1480	37	19	3.1×10^4	9×10^6	GOT 1.5×10^4
Alanine	1960	39	20	3.3×10^4	9.4×10^6	GPT 1.9×10^4

[a] Formula weight (F.W.) 40 000, M. Shin, *Methods Enzymol.*, 1971, **23**, 441. [b] F.W. 2 200 000, H. Surd and W. Burchard, *Eur. J. Biochem.*, 1968, **6**, 202. [c] F.W. 110 000, W. T. Jenkins, D. A. Yphantis, and I. W. Siger, *J. Biol. Chem.*, 1959, **234**, 51. [d] F.W. 115 000, M. Saier, Jr., and W. T. Jenkins, *J. Biol. Chem.*, 1967, **242**, 91.

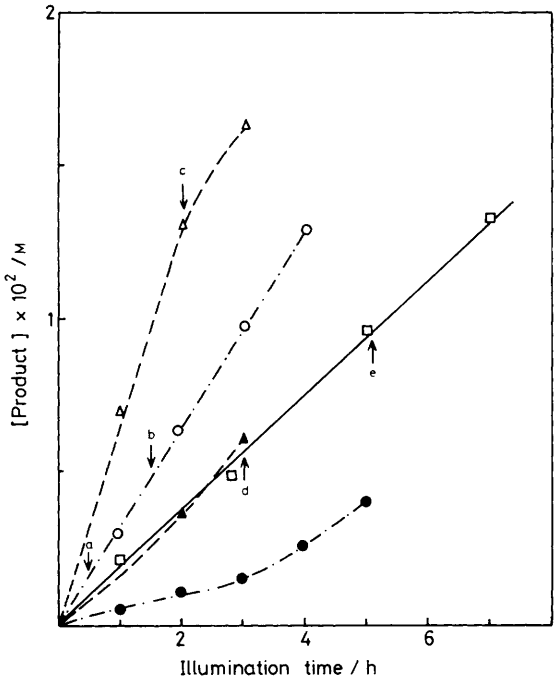

Figure 1. Rate of amino acid formation as a function of illumination time: ○, aspartic, and ●, glutamic acid, in the coupled system (–·–·–·–), a, b, addition of 1×10^{-2} M mercaptoethanol; △, alanine, and ▲, glutamic acid, in the coupled system (– – – –), c, addition of 1×10^{-2} M mercaptoethanol; □, glutamic acid by itself (————), d, e, addition of 1.2×10^{-2} and 1.9×10^{-2} M mercaptoethanol.

the formation of NADPH in the presence of the enzyme ferredoxin-NADP$^+$-reductase (FDR, E.C. 1.18.1.2). The photoinduced regeneration system of NADPH was coupled to secondary enzyme-catalysed processes such as reduction of ketones to alcohols[5,6] and CO_2 fixation.[7] Here, we report on the conjugation of the photo-induced NADPH regeneration cycle to a secondary enzyme-catalysed process forming glutamic acid. This system is coupled to a tertiary 'one-pot' enzyme-catalysed transamination process producing aspartic acid and alanine.

The systems were composed of an aqueous buffer solution (Tris buffer, 0.1 M, pH 7.9) that includes trisbipyridineruthenium dication $Ru(bpy)_3^{2+}$ (2.2×10^{-5} M), as sensitizer, 2-mercaptoethanol (1.75×10^{-2} M) as electron donor, MV^{2+} (2.75×10^{-4} M) as primary electron acceptor, $NADP^+$ (8.7×10^{-4} M), NH_4^+ (8.7×10^{-2} M), and α-oxoglutarate (0.1 M). Two enzymes were introduced into this system: ferredoxin-NADP$^+$-reductase (FDR, E.C. 1.18.1.2, 0.2 units) and glutamic dehydrogenase (GluDH, E.C. 1.4.1.3, 40 units). Illumination of the deaerated system (λ >420 nm) resulted in the formation of glutamic acid (**1**). The rate of glutamic acid formation as a function of illumination time is displayed in Figure 1. The mechanism leading to the formation of (**1**) is schematically presented in Figure 2 and involves the photosensitized regeneration of NADPH acting as the cofactor of the reductive amination of oxoglutarate.

The photoinduced production of glutamic acid allows coupling of the system to tertiary transamination enzymes that produce aspartic acid (**2**) and alanine (**3**). In these systems α-oxoglutaric acid (8.7×10^{-3} M) mediates the production of glutamic acid. Oxaloacetic acid (8.7×10^{-2} M) and the enzyme glutamic–oxaloacetic transaminase (GOT, E.C. 2.6.1.1, 80 units) or pyruvic acid (8.7×10^{-2} M) and glutamic–pyruvic

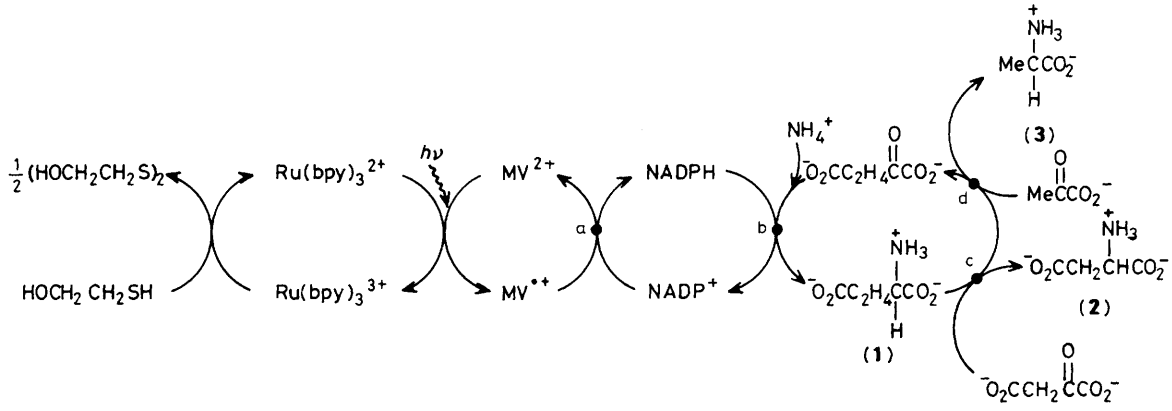

Figure 2. Cyclic scheme for the photoinduced production of amino acids: a, ferredoxin-NADP$^+$-reductase; b, glutamic dehydrogenase; c, glutamic–oxaloacetic transaminase; d, glutamic–pyruvic transaminase.

$$HOCH_2CH_2SH + NH_4^+ + HO_2CCH_2CH_2\overset{O}{\overset{\|}{C}}CO_2H \longrightarrow \tfrac{1}{2}(HOCH_2CH_2S)_2 + HO_2CCH_2CH_2\overset{+}{C}HCO_2H + H_2O \quad (1)$$

$$HOCH_2CH_2SH + NH_4^+ + HO_2C\overset{O}{\overset{\|}{C}}CH_2CO_2H \longrightarrow \tfrac{1}{2}(HOCH_2CH_2S)_2 + HO_2CCH_2\overset{+}{C}HCO_2H + H_2O \quad (2)$$

$$HOCH_2CH_2SH + NH_4^+ + HO_2C\overset{O}{\overset{\|}{C}}Me \longrightarrow \tfrac{1}{2}(HOCH_2CH_2S)_2 + HO_2C\overset{+}{C}HMe + H_2O \quad (3)$$

transaminase (GPT, E.C. 2.6.1.2, 40 units) were introduced into the previously described system. Upon illumination of the deaerated solutions ($\lambda > 420$ nm), a mixture of glutamic acid (**1**) and aspartic acid (**2**) was obtained in the former system and a mixture of (**1**) and alanine (**3**) in the latter system. The content[8] of the amino acids in solution as a function of illumination time is also displayed in Figure 1. The overall reactions accomplished in the NADPH-mediated processes (Figure 1) correspond to the reductive amination of α-oxoglutaric acid, oxaloacetic acid, and pyruvic acid to give the respective amino acids by mercaptoethanol (equations 1—3). The turnover numbers of the different components included in the systems are summarized in Table 1, and correspond to a 19% conversion of oxaloacetic acid and a 20% conversion of pyruvic acid. The results imply that the enzymes exhibit high stability and activity in the artificial environments.

In conclusion, we have demonstrated that the photosensitized NADPH regeneration system can be coupled to amino acids syntheses by enzyme-catalysed processes. The stability of the enzymes and high quantum yields of the NADPH regeneration[5,6] might have practical biotechnological implications.

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