Short Communications

Flavin Mononucleotide-Sensitized Photo-oxidation of Glyoxylate in Good's Buffers

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Subcellular fractions from plant leaves can catalyse the release of CO₂ from the carboxyl groups of glycollate and glyoxylate at pH 8 (Halliwell *et al.*, 1972). However, we found that FMN, a cofactor for these reactions, also induced an appreciable non-enzymic decarboxylation of glyoxylate in certain buffers unless the reaction mixtures were protected from light. This was particularly marked when Good's buffers (Good *et al.*, 1966) were used. The cause of this reaction was investigated.

The non-enzymic decarboxylation was assayed by incubating 15 μ mol of sodium glyoxylate (Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K.) containing 5×10^5 c.p.m. of [1-¹⁴C]glyoxylate (The Radiochemical Centre, Amersham, Bucks., U.K.) with 100 μ mol of buffer adjusted to pH 8 with either HCl (tris) or with KOH [N-glycylglycine, Tes (*N* - tris(hydroxymethyl)methyl - 2 - aminoethane - sulphonic acid), Hepes (2-*N*-hydroxyethylpiperazin-*N'*-ylethanesulphonic acid) and Tricine (*N*-tris(hydroxymethyl)methylglycine)] in a total volume of 3 ml in sealed flasks for 2 h at 25°C with shaking. Where indicated, 0.1 μ mol of FMN was also added. Each flask was fitted with a small plastic tube con-

taining 0.2 ml of 20% (w/v) KOH to collect ¹⁴CO₂. After 2 h, 0.2 ml of 2.5 M-H₂SO₄ was added to each reaction mixture and incubation at 25°C was continued for a further 30 min to drive off CO₂ retained in the solution. Finally, 0.1 ml of the KOH solution was transferred to 5 ml of scintillation fluid and the radioactivity was measured by liquid-scintillation counting. When the acid was added at the beginning of the assay procedure and the flasks were incubated for 2.5 h, no release of CO₂ from glyoxylate was detected whatever buffer was present, indicating that no decomposition occurs during this part of the assay.

The non-enzymic decarboxylation of glyoxylate at pH 8 in glycylglycine buffer is slow whether or not FMN is present (Table 1). When glycylglycine was replaced by tris, non-enzymic decarboxylation was increased slightly, but the use of Good's buffers produced a striking increase, most marked in the case of Hepes (Table 1). This agreed with the results of our enzyme assays on subcellular particles, when the decarboxylation of glyoxylate in controls (for which the extracts had been heated at 100°C) was less than 10% of the enzymic rate in glycylglycine buffer, but was substantially greater than this, often exceeding

Table 1. *Effect of buffers on non-enzymic glyoxylate decarboxylation*

Assays were carried out as described in the text. 'Light' means that the reaction flasks were exposed to normal laboratory light; 'dark' means that the flasks were wrapped in a double layer of aluminium foil to exclude light.

Buffer	Rate of CO ₂ release (nmol/2h)		
	FMN absent (light)	FMN present (light)	FMN present (dark)
N-Glycylglycine	9	11	9
Tris	8	20	8
Tes	7	42	9
Hepes	9	173	8
Tricine	7	118	8

Table 2. *Effect of catalase on non-enzymic glyoxylate decarboxylation*

Assays were carried out as described in the text. All flasks were exposed to normal laboratory light. Where indicated, 1800 units of ox catalase were added.

Buffer	Rate of CO ₂ release (nmol/2h)		
	FMN absent	FMN present	FMN and catalase present
N-Glycylglycine	22	39	32
Tris	22	149	44
Tes	26	179	66
Hepes	23	623	137
Tricine	21	556	155

the enzymic rate, when Good's buffers were substituted. Exclusion of light from the reaction mixtures decreased the decarboxylation to the rate in the absence of FMN (Table 1).

Decarboxylation was markedly diminished by the addition of catalase (Table 2). It therefore seems likely that H_2O_2 is generated in an FMN- and light-dependent reaction. It is known that glyoxylate is rapidly decomposed by peroxide to yield CO_2 (from the carboxyl group) and formate (Davies & Corbett, 1969). Good's buffers have been shown to reduce FMN to FMNH_2 in illuminated systems (Yamazaki & Tolbert, 1970). Reoxidation of FMNH_2 by molecular O_2 would account for the generation of H_2O_2 in our system. Our results therefore suggest that this reduction can take place even in normal laboratory lighting conditions (a typical value for the

light-intensity in our laboratory is 12 lm/m^2). It is therefore likely that the results of experiments in which Good's buffers are used with FMN-containing systems, especially if exposed to strong light, may be seriously affected by the generation of H_2O_2 .

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Davies, D. D. & Corbett, R. J. (1969) *Phytochemistry* **8**, 529

Good, N. E., Winget, G. D., Winter, W., Connolly, T. N., Izawa, S. & Singh, R. M. M. (1966) *Biochemistry* **5**, 467

Halliwel, B., Leek, A. E. & Butt, V. S. (1972) *Biochem. J.* **128**, 87P

Yamazaki, R. K. & Tolbert, N. E. (1970) *Biochim. Biophys. Acta* **197**, 90