Phytochemistry, Vol. 22, No. 2, pp. 347–352, 1983.
Printed in Great Britain.

0031-9422/83/020347-06$03.00/0

INHIBITION OF GLYCOLLATE METABOLISM BY AMINO-OXYACETATE: EFFECTS ON PIGMENT FORMATION IN HIGHER PLANTS

C. L. D. JENKINS*, L. J. ROGERS and M. W. KERR†

Department of Biochemistry, University College of Wales, Aberystwyth, SY23 3DD, U.K.;† Shell Research Limited, Sittingbourne Research Centre, Kent, ME9 8AG, U.K.

(*Received* 26 *April* 1982)

Key Word Index—*Hordeum vulgare*; *Zea mays*; Gramineae; glycollate metabolism; pigment formation; greening; amino-oxyacetate.

Abstract—In greening leaf segments amino-oxyacetate inhibited both chlorophyll and carotenoid formation by *ca* 60% at 0.5 mM inhibitor concentration. In greening tissue serine:glyoxylate aminotransferase was the only enzyme of the glycollate pathway whose activity was markedly decreased after inhibitor treatment. The inhibition of pigment formation in barley and maize could be alleviated by glyoxylate, pyruvate and acetaldehyde; in the latter case there is probably a preferential reaction with inhibitor which displaces it from combination with enzymic pyridoxal 5′-phosphate.

INTRODUCTION

Amongst the roles proposed for the glycollate pathway in higher plants have been suggestions that in greening tissue it can make an appreciable contribution in the formation of chlorophyll [1–3] and chloroplast terpenoids [4]. Experiments with isonicotinyl hydrazide (INH), a photorespiratory inhibitor which may inhibit preferentially the glycine to serine conversion [5, 6], have given support to these views. INH effectively prevented greening of etiolated leaf tissue, and restoration of pigment formation could be achieved by supplementation with intermediates in the glycollate pathway beyond the site of inhibition [7]. It has further been shown that *s*(+)-2-hydroxy-3-butynoic acid (HBA), a specific inhibitor of glycollate oxidase, will also prevent the formation of chloroplast pigments in greening tissue [8, 9].

Amino-oxyacetate (AOA; NH_2OCH_2COOH) is similar to INH in showing general reactivity towards pyridoxal phosphate (PLP)-requiring enzymes. It has been widely used as an aminotransferase inhibitor in studies of intermediary metabolism in animal tissues [10, 11]. Reports of its previous use in plant systems have been limited to studies on ammonia assimilation [12] and use as an inhibitor of glutamine α-oxoglutarate transaminase in an alga [13]. In various of these studies on aspects of intermediary metabolism it has been generally accepted that a readily reversible inhibition is involved, though this view has been questioned [14].

In similar investigations to those reported previously for INH [7] and HBA [8, 9], we have examined the effect of AOA on greening of *Hordeum vulgare* (barley) and *Zea mays* (maize). In interpreting the data obtained, it proved necessary to characterize the interaction of AOA with PLP in the absence or presence of various metabolites, and these studies are also reported.

* Present address: C.S.I.R.O., Division of Plant Industry, P.O. Box 1600, Canberra City, A.C.T. 2601, Australia.

RESULTS AND DISCUSSION

Interaction of AOA and pyridoxal phosphate

The characteristics of interaction of inhibitor with coenzyme were central to our investigations and were studied in some detail. Combination of AOA with PLP is characterized by a change in the absorption spectrum of the coenzyme; the peak at 388 nm decreasing while that at 330 nm increases and shifts to 335 nm; the isosbestic point is at 366 nm (Fig. 1). In the UV region there is a considerable increase in *A* at 266 nm. These data are consistent with spectral changes accompanying reaction between aspartate transaminase and AOA [14] and indicate that the previously published spectrum for the interaction of AOA with PLP [15] is incorrect. Since the *A* peak at 388 nm can be attributed to the presence of the free aldehyde group in PLP (see e.g. ref. [16]) the decreased absorption at this wavelength is consistent with formation of an aldehyde addition compound with AOA to form a substituted oxime. The involvement of the aldehyde group was also supported by the observation that when AOA was incubated with pyridoxamine there was no change in the absorption spectrum of the latter compound.

Bisulphite has been shown to prevent Schiff-base formation between PLP and amino acids, owing to its reaction with the aldehyde group of PLP [17]. In the present study, the PLP–bisulphite addition complex, formation of which was shown by substantially decreased *A* at 388 nm, was obtained by prolonged reaction of 0.2 mM bisulphite with equimolar PLP. Subsequent addition of AOA (1 mM) caused the spectrum characteristic of the AOA–PLP complex to be formed within 45 min. This suggests that the binding between PLP and AOA is a considerably stronger one than the usual Schiff-base linkage formed between PLP and amino acids, other than those with sulphydryl groups. It might be noted that an increased *A* at 335 nm, similar to our observations on the interaction of AOA with PLP, occurred on reaction of

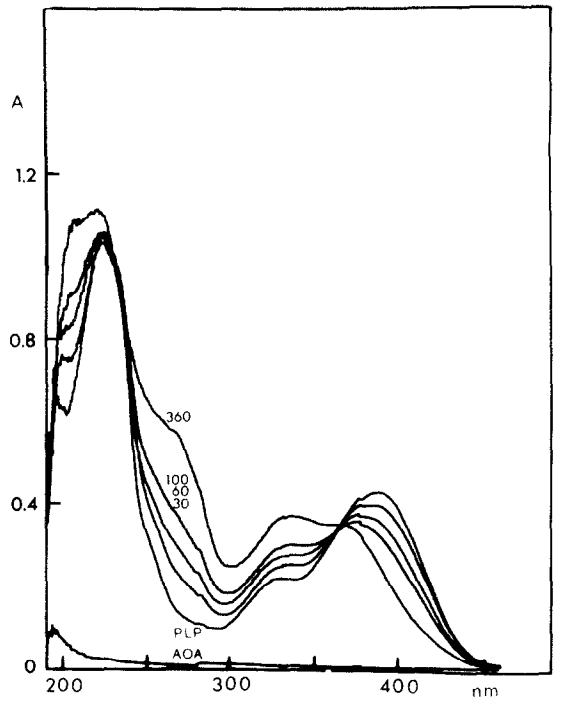

Fig. 1. Absorption spectra after addition of AOA to PLP. Spectra were recorded at the time indicated (min) following addition of AOA to PLP, both at a final concentration of 0.1 mM in 2.5 mM KH_2PO_4, pH 7, at 20°. Also shown are the spectra for each of the reactants alone, at 0.1 mM.

sulphydryl-containing amino acids with PLP to give stable products containing a thiazolidine (cysteine) or thiazine (homocysteine) ring [18].

That the binding of PLP with AOA is substantially stronger than a Schiff-base linkage was confirmed in experiments where equimolar amounts of glycine, AOA and PLP were reacted. Here the absorption spectra recorded after various periods were characteristic of formation of the PLP–AOA complex. If glycine and PLP (both at 0.1 mM) were pre-incubated at room temperature for several hours to facilitate Schiff-base formation, indicated by the *A* band at 388 nm being shifted to longer wavelength [18], addition of AOA (1 mM) resulted in formation within 10 min of the PLP–AOA complex.

From spectrophotometric studies of the reaction of PLP with amino acids and amines, it could be shown that the presence of various metal ions could influence the spectra of the Schiff-bases formed, this being attributed to chelation effects [18]. In contrast, we found PLP–AOA complex formation was unaffected by the presence of 1 mM Mg^{2+}; in this respect also the PLP–AOA complex reaction differs from the usual Schiff-base linkage formed with other amino compounds.

With equimolar (0.1 mM) AOA and PLP in 2.5 mM potassium dihydrogen phosphate, pH 7, reaction at 20° was complete within 6 hr (Fig. 1), and with a 10 or 100-fold excess of AOA reaction was complete within 1 hr and within 4 min, respectively (data not shown). From the final spectra obtained in the last reaction an $\varepsilon_{335\ nm}$ of 4.66 $\times 10^3$ l/mol/cm was derived for the PLP complex, assuming that all the PLP present had reacted. This value, and an $\varepsilon_{388\ nm}$ of 4.9×10^3 l/mol/cm for PLP at the same pH [16] were used to derive a second-order rate constant of 66.3/M/min for complex formation. Essentially the same result was obtained from initial rates of first order (in PLP) semilogarithmic plots of remaining PLP against time at several AOA concentrations. The value obtained is considerably lower than the only other quoted rate constant for this reaction of 290/M/min [19]. The reason for this is unclear, but might be explained by differing experimental conditions since it is known that addition reactions of ammonia derivatives with carbonyl compounds are dependent on precise pH control owing to possible protonation of both the carbonyl oxygen atom and the nitrogen of the ammonia derivative.

Effect of AOA on pigment formation

Studies with INH, which acts in a similar way to AOA, showed that this would inhibit greening of etiolated barley and maize [7]. Supplying either intermediates of the glycollate pathway beyond the site of inhibition, or pyruvate, restored greening, providing evidence which supports proposals [3, 4] that the glycollate pathway may play a role in developing tissues in the formation of chlorophyll and chloroplast terpenoids. These observations prompted similar studies with AOA.

The effects of various concentrations of AOA on pigment formation in etiolated barley and maize are shown in Fig. 2. In both, effective inhibition of greening was observed, with the saturating inhibitor concentration of *ca* 0.7 mM causing decreases in amounts of chlorophyll of *ca* 70% in both cases. Carotenoid concentrations were similarly decreased in barley, and in maize declined to those typical of etiolated tissue. The effective concentration of AOA for these studies was higher than needed to cause appreciable inhibition of carbon dioxide assimilation but it will be noted (see ref. [27]) that experimental conditions are quite different. In the carbon dioxide assimilation experiments, leaf sections were placed in the assimilation chamber in an air stream with their cut bases in solution containing inhibitor, whereas in greening studies leaf segments were floated on the AOA solution; in the latter case the uptake of inhibitor might be far less effective. AOA was *ca* 10 fold more effective in inhibiting greening than INH under similar experimental conditions [7].

Effects on enzymes of the glycollate pathway

Assays *in vitro* of the effect of AOA on enzymes of the glycollate pathway (data in ref. [27]) had suggested that serine:glyoxyate aminotransferase may have been the primary site of action of the inhibitor, though at higher AOA concentrations glutamate: glyoxylate aminotransferase was also affected. We also assayed enzymes of the glycollate pathway in leaf segments used in the greening studies. In this case etiolated leaf segments were incubated in 1 mM AOA for 3 hr before illumination for 48 hr to promote greening. Enzyme amounts were assayed at the end of the experiment and compared with those in etiolated tissues and in tissues greened in the absence of AOA (Table 1). These studies again suggested that serine:glyoxylate aminotransferase was the primary site of inhibition by AOA, though glutamate:glyoxylate aminotransferase was also decreased in some experiments. The potency of AOA compared to the more widely used PLP-dependent enzyme

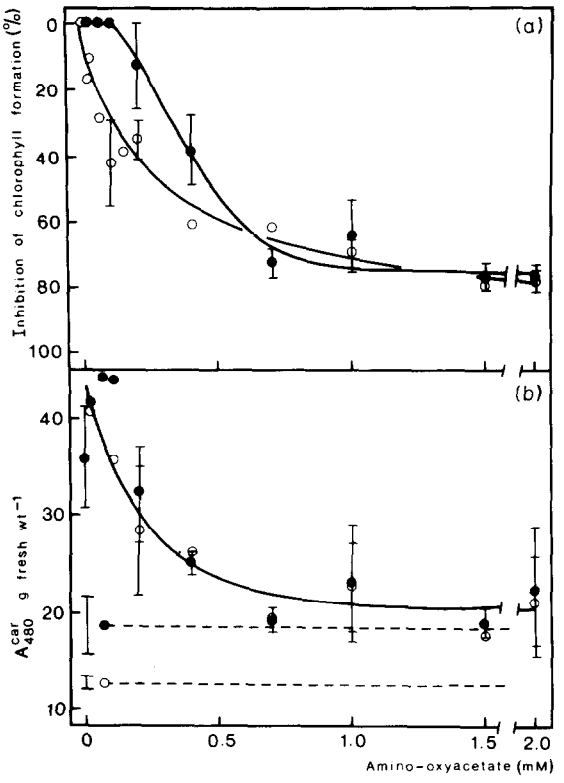

Fig. 2. Inhibition of pigment formation in illuminated etiolated leaf segments by AOA. *Ca* 1 g of etiolated 7-day-old barley or 8-day-old maize leaf segments were floated on 20 ml 2.5 mM KH_2PO_4, pH 7, containing AOA at the concentration indicated. Samples were incubated in darkness for 3 hr then illuminated (*ca* 4 klx) for 48 hr at 26° before chlorophyll and carotenoid estimation; (○) barley; (●) maize. Vertical bars indicate mean ± s.d. for three experiments. (a) Inhibition of chlorophyll formation by AOA. Amount of chlorophyll in the absence of inhibitor was 852 ± 128 ($n = 4$) and 490 ± 64 ($n = 4$) μg/g fr. wt for barley and maize, respectively. (b) Inhibition of carotenoid formation by AOA. (----) indicates mean ± s.d. ($n = 4$) for carotenoid in etiolated tissue as $A_{480\,nm}$/g fr. wt.

inhibitor INH is borne out by the observation (data not shown) that under the same experimental conditions as for Table 1 a 10 fold higher concentration of INH was needed to achieve comparable inhibition of serine:glyoxylate aminotransferase. Activities of the other enzymes assayed were all somewhat decreased by AOA treatment and by about the same extent compared with the controls. For glycollate oxidase and hydroxypyruvate reductase amounts were nevertheless within the range found in a number of studies on normal green seedlings. However, partial inhibition of serine hydroxymethyltransferase by AOA may have occurred, though the lower amounts of this enzyme which are apparently present, make interpretation more difficult. In assays of AOA-treated tissue immediately before illumination, activities of this enzyme and of glutamate:glyoxylate aminotransferase were decreased by *ca* 50% whilst serine:glyoxylate aminotransferase activity could not be detected. The possibility remains that PLP-dependent enzymes in other metabolic pathways, e.g. in formation of δ-aminolevulinic acid for chlorophyll synthesis, may also be subject to inhibition by AOA.

Restoration of greening after AOA treatment

The results of attempts to alleviate inhibition of greening in AOA-treated tissue by metabolite supplementation are given in Table 2. Glyoxylate and pyruvate supplementations overcame inhibition of pigment formation in maize and barley and though the extent was variable, appreciable restoration of pigment formation was consistently observed. In the best individual experiments with barley, chlorophyll formation was restored to *ca* 90% by glyoxylate and fully with pyruvate. In maize, glyoxylate and pyruvate additions gave restoration of chlorophyl formation to maximally 70 and 90%, respectively, that of untreated controls. Carotenoid amounts in both species were also substantially restored by supplementation with either glyoxylate or pyruvate. Supplementations with glycollate, glycine or serine gave no appreciable improvement in greening, though sometimes glycine and serine were slightly beneficial. Following a report [11] that both pyruvate and acetaldehyde could reverse the inhibition by AOA of alanine and aspartate

Table 1. Effect on activities of glycollate pathway enzymes following greening of etiolated barley leaf segments in the presence of AOA

Enzyme	Enzyme activity (μmol/min/g fr. wt)		
	Etiolated	Greened, AOA absent	Greened, AOA present
Glycollate oxidase	1.07	1.34	1.01
Glutamate:glyoxylate aminotransferase	1.21	4.47	< 4.5
Serine:glyoxylate aminotransferase	1.21	1.65	0.30
Serine hydroxymethyltransferase	0.05	0.06	0.04
Hydroxypyruvate reductase	4.90	7.30	5.40

1 g of etiolated 7-day-old barley leaf segments, *ca* 1 cm long, was floated on 20 ml 2.5 mM KH_2PO_4, pH 7, containing 1 mM AOA. Samples were incubated in darkness for 3 hr then illuminated (*ca* 4 klx) for 48 hr at 26° before enzyme estimation. Activities in extracts from etiolated leaves, and in leaf segments from etiolated shoots greened as above but not exposed to inhibitor, are also shown.

Table 2. Effect of metabolite supplementation on the AOA inhibition of chlorophyll formation in illuminated etiolated leaf segments

		Barley		Maize	
		Chlorophyll (μg/g fr. wt)*	% greening	Chlorophyll (μg/g fr. wt)*	% greening
Controls	E.T.†	24 ± 7(8)	0	23 ± 10(6)	0
	G.T.†	649 ± 165(8)	100	557 ± 40(6)	100
	G.T.I.	188 ± 13(4)	30 ± 7(4)	111 ± 16(5)	16 ± 2(5)
Metabolite supplemented (mM)					
Glycollate	1.0	182 ± 52(4)	28 ± 9(4)	136	17
	3.0	194 ± 60(4)	31 ± 11(4)	124	15
Glyoxylate	1.0	344 ± 89(4)	56 ± 14(4)	276	49
	3.0	422 ± 127(4)	69 ± 20(4)	396	72
Glycine	1.0	180 ± 22(4)	27 ± 4(4)	147 ± 26(4)	24 ± 4(4)
	3.0	202 ± 21(4)	32 ± 5(4)	163 ± 42(4)	26 ± 7(4)
Serine	1.0	187 ± 18(4)	29 ± 7(4)	154 ± 34(4)	25 ± 7(4)
	3.0	217 ± 32(4)	34 ± 6(4)	175 ± 49(4)	29 ± 9(4)
Pyruvate	1.0	301 ± 99(4)	48 ± 15(4)	188 ± 64(5)	30 ± 12(5)
	3.0	428 ± 81(4)	72 ± 20(4)	326 ± 114(5)	56 ± 22(5)
Acetaldehyde	1.0	379	75	293	75
	3.0	320	63	326	83

1 g of etiolated 7-day-old barley or 8-day-old maize leaf segments, *ca* 1 cm long, was floated on 20 ml 2.5 mM KH_2PO_4, pH 7, containing AOA and metabolite as indicated. Samples were incubated in darkness for 3 hr then illuminated (*ca* 4 klx) for 48 hr at 26° before pigment estimation. E.T., Etiolated tissue incubated in the dark; G.T., greened tissue incubated in the light; G.T.I., tissue greened in the presence of AOA (0.8 mM for barley, 1 mM for maize).

* Some data are given as mean ± s.d. for *n* experiments.

† These values form the basis for calculation of % greening in each experiment. % greening in the cases of metabolite addition is based on comparison with the actual values obtained in the appropriate experiment. In control experiments greening in maize was unaffected by supplementation with 3 mM pyruvate in the absence of AOA, but in barley was *ca* 15% decreased.

aminotransferases from hepatocytes by complexing more strongly with AOA, thereby freeing the enzymic PLP, supplementations with acetaldehyde were also carried out. In both barley and maize, acetaldehyde was effective in restoring pigment formation (Table 2). In other experiments (data not shown) no greening of AOA-inhibited tissue was observed following supplementations with sucrose, glutamate, formate or various combinations of these metabolites. Acetate also was not effective in restoring greening. Though chloroplastidic acetyl coenzyme A synthetase initiates its ready incorporation into fatty acids in green leaves [20], it does not appear to support chloroplast terpenoid biosynthesis in etiolated tissues undergoing greening (see e.g. ref. [4]). Hydroxypyruvate and glycerate appeared to restore greening to some extent in both barley and maize, possibly through their metabolism to pyruvate.

The observations that glyoxylate, pyruvate and acetaldehyde would restore greening in AOA-treated tissue prompted further *in vitro* spectrophotometric studies. In these the abilities of pyruvate (ketone), or glyoxylate and acetaldehyde (aldehydes), to disturb the interaction between PLP and AOA were tested. Reaction components throughout were in 2.5 mM potassium dihydrogen phosphate, pH 7, and were at 20°. When equimolar amounts of pyruvate or glyoxylate, AOA and PLP were mixed, the spectrum characteristic of the AOA–PLP complex was formed. The second-order rate constant obtained was similar to that in the absence of metabolite, indicating that pyruvate and glyoxylate do not prevent complex formation. However, when acetaldehyde was present in the mixture with PLP and AOA the characteristic PLP spectrum remained unchanged, indicating that in this case AOA had preferentially reacted with the acetaldehyde instead of the coenzyme. When PLP was incubated with AOA for several hours before metabolite was added, there was no change in the absorption spectrum over a 3 hr period with any of the three metabolites, confirming that, *in vitro* at least, the metabolites were unable to dissociate AOA from its combination with the coenzyme.

Possible reactions between these metabolites and AOA were further examined by incubating them with an equimolar amount of AOA for several hours before adding PLP and recording the resulting spectrum after various periods. The results for preincubation of AOA with glyoxylate or pyruvate were similar, the spectrum of the PLP–AOA complex being gradually formed, but less rapidly than in absence of preincubation with metabolite. Consistent with this, second-order rate constants of 22.2 and 26.6/M/min, respectively, were determined. In nei-

ther case, however, had the reaction proceeded to completion in 20 hr. After preincubation of AOA with acetaldehyde, complex formation between AOA and subsequently added PLP was negligible.

The question whether the observed restoration of greening by glyoxylate, pyruvate or acetaldehyde resulted from reversal of enzyme inhibition is, therefore, unresolved though the balance of evidence suggests that the first two metabolites restored greening as a result of their subsequent metabolism. This conclusion in the case of pyruvate is supported by the recent report [21] (cf. ref. [11]) that in isolated hepatocytes or cell extracts, pyruvate did not block or reverse the inhibition of aspartate aminotransferase by AOA.

Thus, in the present studies, the activities of PLP-dependent enzymes were considerably decreased in etiolated tissue after AOA treatment or after greening in the presence of AOA (Table 1), and these inhibitions were not alleviated by PLP in the assay mixtures. This suggests that AOA becomes strongly complexed to the enzymes via their bound coenzyme. Similarly, enzyme inhibition *in vitro* was substantial (Fig. 1), and in the case of serine:glyoxylate aminotransferase was unaffected by the presence of glyoxylate as a substrate.

Spectrophotometric experiments on the interaction of PLP with AOA were also consistent with strong interaction to form a stable complex, comparable with that observed for the sulphydryl-containing amino acids, cysteine and homocysteine [18], rather than the weaker Schiff-base type linkage formed between PLP and most other amino compounds. Most convincingly, this reaction between AOA and PLP was unaffected by including equimolar glyoxylate or pyruvate in the reaction mixture, and still occurred, although less rapidly, after preincubation of these metabolites with AOA for several hr. However, similar experiments with acetaldehyde showed that in this case the reaction of AOA with PLP was prevented. None of the three metabolites would react with the pre-formed AOA–PLP complex.

These *in vitro* reactions of PLP with AOA may not adequately represent the situation where AOA reacts with enzyme-bound PLP, where AOA binding could be less effective. However, the reports of other authors suggest that enzyme-bound PLP is more, rather than less, susceptible to AOA inhibition. Thus second-order rate constants of 3700 and 1000/M/min were derived [19] for the biphasic portions of the reaction of AOA with the PLP-dependent enzyme, cystathionase, compared with a value of 290/M/min for the reaction between AOA and free PLP under the same conditions. It was suggested that in the former case, the Schiff-base linkage between the PLP and enzyme might confer a direct kinetic advantage during the reaction between the cofactor and the substrate (or AOA). Second-order rate constants of 1300 and 400/M/sec have been determined for the reaction of AOA with 4-aminobutyrate transaminase and aspartate transaminase, respectively [14], and while the complex formation in the first reaction proved irreversible, the combination of aspartate transaminase with AOA could be partly reversed only by addition of a more reactive inhibitor (cysteinesulphinate). Subject to the uncertainties implicit in studies of inhibitor action, these interpretations of the interactions of PLP with AOA suggest that in AOA-inhibited leaves, glyoxylate and pyruvate may act as percursors for terpenoids in greening tissue. While pyruvate incorporation in greening tissues might be explained by its involvement in the final stages of a glycollate to acetyl-CoA pathway, the reason for glyoxylate utilization remains unsolved.

EXPERIMENTAL

Chemicals. Amino-oxyacetic acid (carboxymethoxylamine) was obtained from Aldrich Chemicals, as a hemihydrochloride and solns were neutralized before use with KOH. Tetrahydrofolic acid solns were prepared in de-aerated 0.1% (v/v) 2-mercaptoethanol and neutralized with NaOH under N_2.

Growth of seedlings. Seeds of *Hordeum vulgare* var. Mazurka were obtained from the Welsh Plant Breeding Station, Aberystwyth, U.K., and seeds of *Zea mays* var. Dekalb 202 were supplied by Miln Masters Ltd., Chester, U.K. Etiolated seedlings were grown in vermiculite or soil in an environmental growth chamber in darkness at 25°. For normal (green) seedlings growth was under a 14 hr regime (day temp. 26°, night temp. 20°) with fluorescent and tungsten lights providing 8 klx at the leaf surface.

Greening studies. As described in ref. [8].

Enzyme assays. A known wt of leaf material (0.2–1 g) in 10 ml 0.1 M Tris–HCl, pH 8.2 (glycollate oxidase), 0.1 M KH_2PO_4, pH 7 (glutamate:glyoxylate aminotransferase and serine hydroxymethyltransferase), or 0.1 M Hepes, pH 7 (serine:glyoxylate aminotransferase and hydroxypyruvate reductase), was thoroughly homogenized at *ca* 4° by several 5–10 sec bursts with an Ultra-Turrax homogenizer. The homogenate was centrifuged at *ca* 2000 *g* for 15 min and the supernatant was recovered. Determinations of protein in extracts by the Folin–phenol method showed that 1 g fr. wt green tissue was equivalent to *ca* 30 mg soluble protein, while etiolated tissue yielded half this amount. To allow comparisons, results are usually expressed on a fr. wt basis.

Glycollate oxidase assays based on the method of ref. [22] were carried out in an O_2 electrode at 30°. The reaction mixture (3.2 ml) contained: Tris–HCl buffer (pH 8.2), 150 μmol; FMN, 0.2 μmol; sodium azide (to inhibit any residual catalase activity), 3 μmol; sodium glycollate, 3 μmol; and generally 1 ml of extract containing 1–2 mg protein, though this varied depending on activity.

Glutamate:glyoxylate aminotransferase was assayed by a radiochemical method [23, 24]. The reaction mixture (1.1 ml) at 30° contained: KH_2PO_4 (pH 7.0), 65 μmol; PLP, 0.1 μmol; L-glutamate, 30 μmol; [1-^{14}C]glyoxylate, 10 μmol (0.5 μCi); and 0.2 ml of plant extract. The reaction was started by addition of [1-^{14}C]glyoxylate, and terminated after 10 min by heating to 100° for 2 min. After cooling, the pptated protein was removed by centrifugation at *ca* 2000 *g* for 10 min. The product of the reaction, confirmed as [1-^{14}C]glycine by chromatography, was separated from excess [1-^{14}C]glyoxylate by ion exchange chromatography on a column (5.5 × 1.0 cm) of Dowex 1-X8 (acetate form) and its radioactivity assayed.

Serine:glyoxylate aminotransferase was assayed spectrophotometrically by NADH oxidation in a coupled system with hydroxypyruvate reductase [24, 25]. The reaction mixture (3 ml) at 25° contained: Hepes buffer (pH 7), 200 μmol; NADH, 0.2 μmol; PLP, 0.3 μmol; sodium glyoxylate, 3 μmol; hydroxypyruvate (glyoxylate) reductase, 0.15 units; and usually 0.2 ml of plant extract. A slow endogenous NAD oxidation was observed due to reduction of glyoxylate by the added hydroxypyruvate reductase. After 90 sec the aminotransferase reaction was initiated by addition of 50 μmol L-serine. Since for hydroxypyruvate reductase the K_m for hydroxypyruvate is at least 100-fold lower than that for glyoxylate [24], the increased rate of NADH oxidation observed can be attributed to the formation from serine of hydroxypyruvate. A molar extinction coefficient,

$\varepsilon_{340\,nm}$ of 6.22×10^3 l/mol/cm for NADH was used in calculations.

Serine hydroxymethyltransferase activity was assayed by a radiochemical method based on ref. [26], in which [2-^{14}C]glycine reacts with N^5,N^{10}-methylene tetrahydrofolic acid, generated from 5,6,7,8-tetrahydrofolic acid (THFA) and excess HCHO, to yield radioactive serine and THFA. The reaction mixture (1 ml) at 30° contained: KH_2PO_4 (pH 7.0), 65 μmol; PLP, 0.1 μmol; $MgCl_2$, 3 μmol; THFA, 1.2 μmol; 2-mercaptoethanol, 0.14 μmol; HCHO, 6 μmol; [2-^{14}C]glycine, 5 μmol (0.25 μCi); and 0.2 ml of plant extract. Reaction was initiated by addition of [2-^{14}C]glycine and terminated after 60 min by adding 0.5 ml 10% (w/v) trichloroacetic acid. Pptated protein was removed by centrifugation at 2000 *g* for 10 min. Reaction products in 0.1 ml supernatant were separated by PC on Whatman 3 MM paper with *n*-BuOH–Me_2CO–H_2O–diethylamine (20:20:10:3), as solvent, and zones corresponding to serine were assayed for radioactivity by liquid scintillation in N260 fluid.

Hydroxypyruvate reductase was assayed by following the decrease in $A_{340\,nm}$ due to oxidation of NADH. The reaction mixture (3 ml) at 25° contained: Hepes buffer (pH 7), 200 μmol; NADH, 0.2 μmol; and 0.2 ml of plant extract. A slow endogenous rate was measured over a 90 sec period before starting the reaction by addition of 2 μmol hydroxypyruvate. Calculations assumed a molar extinction coefficient for NADH as before.

Acknowledgements—We thank D. Froggatt for collaboration in spectrophotometric studies and M. M. Williams for technical assistance. C.L.D.J. was in receipt of a SRC-CASE studentship.

REFERENCES

1. Kaler, V. L., Klinger, Yu. E., Loktev, A. V. and Vecher, A. S. (1977) *Sov. Plant Physiol.* **24**, 21.
2. Hendry, G. A. F. and Stobart, A. K. (1977) *Phytochemistry* **16**, 1567.
3. Hendry, G. A. F. and Stobart, A. K. (1978) *Phytochemistry* **17**, 69.
4. Shah, S. P. J. and Rogers, L. J. (1969) *Biochem. J.* **114**, 395.
5. Kumarasinghe, K. S., Keys, A. J. and Whittingham, C. P. (1977) *J. Exp. Botany* **28**, 1163.
6. Servaites, J. C. and Ogren, W. L. (1977) *Plant Physiol.* **60**, 461.
7. Gore, M. G., Hill, H. M., Evans, R. B. and Rogers, L. J. (1974) *Phytochemistry* **13**, 1657.
8. Jenkins, C. L. D., Rogers, L. J. and Kerr, M. W. (1982) *Phytochemistry* **21**, 1849.
9. Jenkins, C. L. D., Rogers, L. J. and Kerr, M. W. (1982) *Phytochemistry* **21**, 1858.
10. Longshaw, I. D., Bowen, N. L. and Pogson, C. I. (1972) *Eur. J. Biochem.* **25**, 366.
11. Smith, S. B., Briggs, S., Triebwasser, K. C. and Freedland, R. A. (1977) *Biochem. J.* **162**, 453.
12. Anderson, J. W. and Done, J. (1977) *Plant Physiol.* **60**, 504.
13. Miflin, B. J. and Lea, P. J. (1975) *Biochem. Biophys. Res. Commun.* **64**, 856.
14. John, R. A., Charteris, A. and Fowler, L. J. (1978) *Biochem. J.* **171**, 771.
15. Mardashev, S. R., Siomina, L. A., Dabagov, N. S. and Goncher, A. R. (1968) in *Pyridoxal Catalysis: Enzymes and Model Systems* (Snell, E. E., Braunstein, A. E., Severin, E. S. and Torchinsky, Yu. M., eds.) pp. 451–466. Wiley Interscience, New York.
16. Johnson, R. J. and Metzler, D. E. (1970) *Methods Enzymol.* **182**, 433.
17. Blakley, R. L. (1955) *Biochem. J.* **61**, 315.
18. Matsuo, Y. (1957) *J. Am. Chem. Soc.* **79**, 2011.
19. Beeler, T. and Churchich, J. E. (1976) *J. Biol. Chem.* **251**, 5261.
20. Murphy, D. J. and Stumpf, P. K. (1981) *Arch. Biochem. Biophys.* **212**, 730.
21. Cornell, N. W., Crow, K. E. and Whitefoot, R. P. (1981) *Biochem. J.* **198**, 219.
22. Kerr, M. W. and Groves, D. (1975) *Phytochemistry* **14**, 359.
23. Kisaki, T. and Tolbert, N. E. (1969) *Plant Physiol.* **44**, 242.
24. Rehfeld, D. W. and Tolbert, N. E. (1972) *J. Biol. Chem.* **247**, 4803.
25. Brock, B. L. W., Wilkinson, D. A. and King, J. (1970) *Can. J. Biochem.* **48**, 486.
26. Cossins, E. A. and Sinha, S. K. (1966) *Biochem. J.* **101**, 542.
27. Jenkins, C. L. D., Rogers, L. J. and Kerr, M. W. (1983) *Phytochemistry* **22**, 19.