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INHIBITION OF GLYCOLLATE METABOLISM BY AMINO-OXYACETATE: CONSEQUENCES FOR PHOTOSYNTHESIS

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 Ltd , Sittingbourne Research Centre, Kent, ME9 8AG, U K

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Abstract—The potential of amino-oxyacetate as an inhibitor of photorespiratory metabolism has been assessed In the presence of inhibitor at 10 μM, assimilation of $^{14}CO_2$ in *Hordeum vulgare* was decreased by 72 %, and in *Zea mays* by 42 %, for amino-oxyacetate at 100 μM the corresponding inhibitions were 90 % and 72 %, respectively Labelling patterns showed reduced incorporation into most metabolites, that into sucrose being notably depressed As a percentage of total incorporation hexose monophosphate, glycollate and serine concentrations were increased following inhibitor treatment The increased incorporation into glycollate was particularly evident in maize, where a marked increase in net assimilation into this compound was observed Enzymic studies on plant extracts treated *in vitro* with amino-oxyacetate suggested that serine glyoxylate aminotransferase was the primary site of inhibition, and glutamate glyoxylate aminotransferase and particularly serine hydroxymethyltransferase were appreciably less sensitive to inhibition

INTRODUCTION

On the view that photorespiration is essentially a wasteful process, one of the methods suggested for its control has been the use of inhibitors of glycollate metabolism [1] The inhibitors investigated have included the aldehyde bisulphite addition compound, α-hydroxy-2-pyridine methanesulphonic acid [1–4], the acetylenic substrate analogue 2-hydroxy-3-butynoic acid (HBA) [5–9], isonicotinic acid hydrazide (INH) [10, 11] and glycidate [12] The first two inhibitors act on glycollate oxidase and HBA in particular is specific for this enzyme The other inhibitors affect later reactions of the glycollate pathway The site of action of glycidate is probably glutamate glyoxylate aminotransferase [13], claims that use of this inhibitor stimulated photosynthesis [12] have since been questioned [6, 14] INH is an inhibitor of enzymes which are pyridoxal 5'-phosphate (PLP)-dependent, and in the glycollate pathway in plants it appears to inhibit preferentially the glycine to serine conversion (see e g refs [3, 6]) More recently glycine hydroxamate [15] and amino-acetonitrile [16] have been shown to inhibit glycine decarboxylation

We now report a study of the inhibition by the carbonyl reagent amino-oxyacetate (AOA) of the glycollate pathway in C_3 (*Hordeum vulgare*) and C_4 (*Zea mays*) plant types Our data suggest AOA may inhibit preferentially serine glyoxylate aminotransferase and is thus a useful addition to the inhibitors currently used for investigating photorespiratory metabolism

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

RESULTS

Effect on photosynthetic carbon dioxide assimilation

An equilibration period of at least 60 min illumination in the leaf chamber of the assimilation apparatus was allowed before exposure to $^{14}CO_2$ to ensure that steady-state photosynthesis was attained in leaf sections Under these conditions the net assimilation rates for untreated seedlings were 12 6 mg CO_2/dm^2/hr for maize and 6 7 mg CO_2/dm^2/hr for barley When experiments on long-term photosynthesis in $^{14}CO_2$–air were carried out using AOA at 0 01 or 0 1 mM the total assimilation of $^{14}CO_2$ into water-soluble material in barley and maize (Table 1) was substantially decreased, with barley appearing to be more susceptible than maize The considerable inhibition of ^{14}C-assimilation that was observed following treatment with the lower AOA concentration emphasizes the potency of this inhibitor

Effect on radioactivity in intermediates

The pattern of ^{14}C-labelling in metabolites in the water-soluble extracts following 30 min photosynthesis in $^{14}CO_2$–air was examined (Table 2) The results for barley indicated lowered incorporation into most metabolites following AOA treatment, with that into sucrose being markedly decreased

After treatment with 0 01 mM AOA an increased incorporation of ^{14}C into glycollate occurred, and a large decrease in glycine These data are consistent with inhibition of the transamination of glyoxylate to glycine, though incorporation into serine as a percentage of total incorporation was increased The decreased incorporation into alanine and aspartate might be due to inhibition

Table 1 Effects of AOA on the net ^{14}C-assimilation into water-soluble material of barley and maize leaf sections during photosynthesis in $^{14}CO_2$–air

	Barley		Maize	
	^{14}C taken up (10^{-5} × dpm)	% decrease	^{14}C taken up (10^{-5} × dpm)	% decrease
No inhibitor	300	—	644	—
AOA 0 01 mM	82	72 7	377	41 5
0 1 mM	30	90 0	178	72 4

Four primary leaf sections of barley or two of maize (total area *ca* 10 cm^2), from 2-week-old seedlings, were arranged in frames with the cut bases in water or solution as indicated, and illuminated (5 klx, temp 25°) and flushed with normal air for 60 min, then with $^{14}CO_2$–air containing 25 $\mu Ci\ ^{14}C/l$ for 30 min (both at 0 8 l/min) before rapid killing, extraction and estimation of ^{14}C in water-soluble material

of the aminotransferases concerned or to inadequate supply of keto-acid precursors from 'respiratory' intermediates, the latter would also account for the decreased ^{14}C-incorporation into malate The severely reduced incorporation of ^{14}C in glycerate could indicate that its formation, via hydroxypyruvate arising from transamination of serine, was inhibited, and this would be consistent with the higher percentage accumulation of ^{14}C in serine Glycerate might also be formed from 3-phosphoglyceric acid, incorporation of ^{14}C into the latter was also inhibited by AOA The data taken as a whole indicate that AOA may affect metabolism in a number of ways, but in the pathway of glycollate metabolism a preferential inhibition of serine glyoxylate aminotransferase appears to occur

After treatment at the higher inhibitor concentration, ^{14}C incorporation into several compounds was virtually abolished In this case the radioactivity in the compounds investigated accounted for only 55 % of the total ^{14}C incorporated, while the remainder was associated with two areas in the phosphate ester region of the developed TLC plate Comparison with published R_f values suggests these compounds may be one or more of erythrose-4-phosphate, xylulose-5-phosphate, ribulose-5-phosphate, sedoheptulose-1,7-diphosphate and sedoheptulose-7-phosphate If phosphate ester intermediates of the Calvin cycle are accumulated this might suggest a direct blockage of photosynthesis by high concentrations of AOA The incorporation of ^{14}C into the hexose monophosphate region also increased as a percentage of the total ^{14}C assimilated after AOA treatment The possibility of direct interaction with sugar monophos-

Table 2 Effect of AOA on the ^{14}C-assimilation into water-soluble products of barley or maize leaf sections following photosynthesis in $^{14}CO_2$–air

	Barley (^{14}C taken up as 10^{-5} × dpm)			Maize (^{14}C taken up as 10^{-5} × dpm)		
Treatment	No inhibitor	0 01 mM AOA	0 1 mM AOA	No inhibitor	0 01 mM AOA	0 1 mM AOA
Total ^{14}C taken up	300 0	82 2	30 2	643 6	376 9	177 8
Intermediates						
3-Phosphoglyceric acid	6 6 (2 2)*	1 0 (1 2)	Neg (<0 1)	26 8 (4 2)	8 5 (2 3)	3 8 (2 1)
Hexose monophosphates	9 3 (3 1)	3 8 (4 6)	1 6 (5 3)	45 3 (7 0)	38 2 (10 1)	20 0 (11 3)
Hexose diphosphates	0 6 (0 2)	0 5 (0 6)	Neg (<0 1)	1 7 (0 3)	2 6 (0 7)	1 4 (0 8)
Sucrose	134 7 (45 0)	23 6 (28 7)	5 0 (16 4)	341 0 (53 0)	150 9 (40 0)	52 7 (29 6)
Glycollate	1 2 (0 4)	1 9 (2 3)	0 4 (1 2)	Neg (<0 1)	49 1 (13 0)	46 9 (26 4)
Glycine	26 8 (8 9)	4 2 (5 1)	0 9 (2 8)	6 6 (1 0)	2 9 (0 8)	Neg (<0 1)
Serine	20 2 (6 8)	17 8 (21 6)	3 9 (12 8)	10 3 (1 6)	9 4 (2 5)	2 6 (1 4)
Glycerate	5 7 (1 9)	0 1 (0 2)	Neg (<0 1)	6 5 (1 0)	Neg (<0 1)	Neg (<0 1)
Alanine	4 2 (1 4)	1 3 (1 5)	0 3 (0 8)	16 3 (2 5)	7 2 (1 9)	Neg (<0 1)
Malate	9 7 (3 3)	2 3 (2 8)	2 6 (8 5)	89 4 (13 9)	48 4 (12 9)	9 7 (5 4)
Aspartate	15 8 (5 2)	2 6 (3 2)	2 0 (6 8)	30 1 (4 7)	9 2 (2 4)	14 8 (8 3)

Experimental details were as for Table 1

Neg, Negligible

Figures in parentheses are ^{14}C present as a percentage of the total taken up

* Incorporations are the means of two experiments, in both cases total ^{14}C incorporation was the same

phates has also been suggested for INH [17] An inhibition of photosynthesis in this way might contribute to the rapid and severe effects of AOA on ^{14}C-assimilation and this aspect is the subject of continuing investigations

A similar picture emerged when maize was treated with AOA, though there were some differences These results (Table 2) show that inhibition of photosynthetic ^{14}C-assimilation was less severe than in barley but was again reflected in decreased incorporation of ^{14}C into phosphoglycerate and sucrose Incorporation into hexose monophosphates and diphosphates increased as a percentage of the total ^{14}C incorporated A small accumulation of ^{14}C into the phosphate ester region was evident in autoradiograms, consistent with earlier results with barley again suggesting that there might be some direct effect of AOA on operation of the Calvin cycle, in the bundle sheath chloroplasts, but these effects were less dramatic than in barley Incorporation of ^{14}C into malate, the C_4-acid playing a critical role in maize photosynthesis, was decreased following AOA treatment, but at the lower AOA concentration the incorporation into this compound was unaffected as a percentage of the total, suggesting no direct effect of AOA on the C_4 pathway Aspartate was also decreased at the lowest AOA concentration, though after treatment with 0 1 mM AOA incorporation of ^{14}C in this compound increased as a percentage of the total This was presumably due to AOA inhibition of aminotransferase activity, but could possibly indicate a greater importance of this acid in C_4 metabolism under the conditions of the experiment Consistent with these views, it has subsequently been shown [Jenkins, C L D unpublished data] that *in vitro* AOA effectively inhibits maize leaf aspartate aminotransferase, but has negligible effects on the enzymes of the C_4 pathway

There were marked effects on ^{14}C-incorporation into intermediates of the glycollate pathway following AOA treatment of maize, with large incorporations of ^{14}C into glycollate With 0 01 mM AOA incorporation of ^{14}C into glycine was somewhat depressed These observations suggest, as for barley, a partial inhibition of glyoxylate aminotransferases involved in the conversion of glycollate into glycine though here incorporation into serine was comparable to the control At 0 1 mM AOA negligible [^{14}C]glycine was formed, and the amount of [^{14}C]serine was also markedly decreased Incorporation of ^{14}C into glycerate was negligible at both inhibitor concentrations, suggesting that in maize ^{14}C incorporated into glycerate comes predominantly from serine rather than from phosphoglycerate, which was still formed to some extent Accumulation of ^{14}C in alanine was also markedly decreased by 0 01 mM AOA treatment, and was negligible with 0 1 mM AOA, again presumably owing to inhibition of aminotransferases In maize too, therefore, the data indicate that AOA inhibition of metabolism may be complex

Effect on enzymes of the glycollate pathway

Although at higher concentrations other effects on photosynthesis are likely, it is apparent that AOA is an inhibitor of photorespiratory metabolism in both C_3 and C_4 plant types The increased incorporation of radioactivity in glycollate, observed particularly in maize, accompanied by the decreased incorporation into glycine would be consistent with inhibition of the PLP-dependent glyoxylate aminotransferases While recognizing that the effects of AOA on other transaminases might have implications for carbon dioxide fixation it was of obvious interest to assess the effect of AOA on enzymes of the glycollate pathway For the present *in vitro* studies of the effects of AOA on individual enzymes PLP was not added to the assays The inhibitions of PLP-dependent glycollate pathway enzymes produced *in vitro* by a range of AOA concentrations is shown in Fig 1 Serine hydroxymethyltransferase is the least affected by the inhibitor, activities being only decreased by some 50% at 1 mM concentration of inhibitor AOA was considerably more potent with the two aminotransferases with glutamate glyoxylate aminotransferase being 80% inhibited by 0 01 mM AOA, while serine glyoxylate aminotransferase activity was abolished at only 0 1 μM and was *ca* 50% inhibited by 0 01 μM inhibitor AOA also inhibited hydroxypyruvate reductase *in vitro* by 30% at 10 mM, but glycollate oxidase was unaffected even at this high concentration

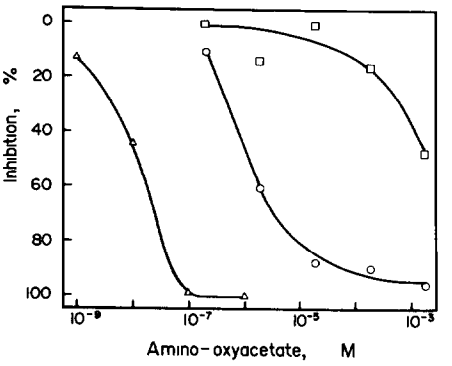

Fig 1 Inhibition of PLP-dependent glycollate pathway enzymes in barley by AOA Activities in extracts of glutamate glyoxylate aminotransferase (○), serine glyoxylate aminotransferase (△) and serine hydroxymethyltransferase (□) were assayed in the presence of AOA added at the concentration indicated before initiation of the reactions by substrate addition Enzyme activities in uninhibited controls, in the absence of supplemented PLP, were 58, 56 and 7 7 nmol/min/mg protein, respectively

In these experiments AOA was added to enzyme before the enzymic reactions were initiated When AOA, to a final concentration 0 1 μM, was added to assays during the course of the reaction serine glyoxylate aminotransferase activity was abolished within 60 sec It is, therefore, likely that in the *in vitro* studies maximum inhibition at each AOA concentration had been attained before initiation of the reactions Tight binding of AOA via enzyme-bound PLP is indicated for serine glyoxylate aminotransferase even in the presence of considerably higher concentrations of serine and glyoxylate

As well as direct consequences for glycollate metabolism there is the possibility of further disturbances in metabolism as a result of inhibition of other PLP-dependent enzymes Of particular interest are the transaminases involved in the regeneration of 2-oxoglutarate in the photorespiratory nitrogen cycle [18] for reassimilation of the ammonia released on conversion of glycine to serine If these were inhibited as a result of AOA treatment

the accumulation of ammonia might cause suppression of carbon dioxide assimilation [19] However, even at the highest inhibitor concentration there was no evidence to suggest that amounts of glutamate or glutamine in AOA-treated plants were significantly different to the controls

DISCUSSION

In interpreting the effects of AOA on carbon dioxide assimilation the data obtained with photorespiratory mutants of *Arabidopsis thaliana* [20] are of particular interest In mutants totally lacking serine glyoxylate aminotransferase, serine and glycine increased as a percentage of total carbon dioxide assimilated, mostly at the expense of starch and sucrose, though 3-phosphoglycerate was also decreased In the studies with 0 01 M AOA (Table 2) the accumulation of serine, and the decrease in phosphoglycerate, are comparable to data for the mutants However, in the studies with the inhibitor there was some decrease in glycine in contrast to data for the *Arabidopsis* mutants Together with the accumulation of glycollate seen following treatment with AOA this suggests partial inhibition of the formation of glycine These additional effects of AOA complicate the changes in metabolic patterns seen after inhibitor treatment The *Arabidopsis* mutants exhibited normal photosynthesis under conditions which suppressed photorespiration and it would be of interest in subsequent studies with AOA to investigate its effect on carbon dioxide assimilation in maize under non-photorespiratory conditions

The potency of AOA is clearly evident when these data are compared with those obtained with the widely used PLP-dependent enzyme inhibitor INH, where considerably higher concentrations are needed to inhibit photorespiratory metabolism to the same extent as AOA (see e g refs [3, 6])

Inhibition of glutamate glyoxylate aminotransferase in tobacco callus [21] and inhibition of this enzyme and serine glyoxylate aminotransferase in leaf discs [13] occurred with the photorespiratory inhibitor glycidate This inhibitor was suggested to inhibit glycollate formation by some unrecognized means through the accumulation in the tissue of glyoxylate and glutamate [22], which apparently inhibited glycollate synthesis and photorespiration [23–25] It is, therefore, of interest that in the present investigations AOA, which also inhibited the aminotransferases, did not result in significant accumulation of glycollate in barley, in contrast to the accumulation observed in AOA-treated maize This observation perhaps indicates a similar inhibition of glycollate formation in barley as a result of glyoxylate aminotransferase inhibition In this case, however, the (possible) inhibition of glycollate synthesis did not result in an increased net carbon dioxide assimilation, in contrast to the results with glycidate [12, 21, 25, 26] Rather, AOA was a rapid and potent inhibitor of net photosynthesis (Table 1) $^{14}CO_2$ assimilation experiments suggested that at the highest AOA concentration there might be a direct effect of AOA on the Calvin cycle, leading to an accumulation of radioactivity into unidentified compounds, possibly phosphate esters Although this may be due to inhibition of other enzyme(s) in the cycle, the possibility that accumulated glyoxylate and/or glutamate were responsible, as suggested elsewhere [22], should be considered In barley this inhibition of photosynthesis might have been the cause of the decreased incorporation of radioactivity in glycollate, rather than vice versa

Further analogies of the effects of AOA and glycidate can be drawn from experiments where glycidate was administered to wheat leaf sections [6] in similar experiments to those reported here for AOA These showed, in agreement with the effects noted for AOA (but cf refs [12, 21, 26]) that glycidate strongly inhibited photosynthesis Concomitant with this was a slightly increased incorporation of $^{14}CO_2$ into glycollate, a large accumulation of phosphate esters and substantial decreases in glycine and serine Incorporation into sucrose was not, however, significantly affected These changes were interpreted as indicating an inhibition of glycollate formation and hence its conversion into subsequent metabolites, possibly due to effects on either ribulose bisphosphate carboxylase or phosphoglycollate phosphatase Nevertheless, the similarity of the effects caused by glycidate and AOA, combined with the knowledge that both compounds can inhibit aminotransferase reactions of the glycollate pathway, are compelling

EXPERIMENTAL

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 Seedlings were grown in vermiculite or soil in an environmental growth chamber under a 14 hr regime (day temp 26°, night temp 20°) with fluorescent and tungsten lights providing 8 klx at the leaf surface

$^{14}CO_2$ *assimilation studies* As described in refs [8, 9] and based on refs [6, 27–29]

Other methods Sources of chemicals and details of enzyme assays are given in the accompanying paper [30]

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