to light grown are in agreement with some other reports (Oelze-Karow et al., 1970, 1976). However, they contradict the opposite findings of Douillard and Bergeron (1978) in young wheat leaves where high lipoxygenase activity was noted in light-grown plants and low enzyme activity in etiolated plants. Zimmerman and Vick (1970) also reported that in mung bean seedlings the lipoxygenase activity in light- and dark-grown seedlings is similar. However, all these reports, except our present study, refer to the activity of lipoxygenase in crude extracts, and no attempt was made to isolate and purify the lipoxygenase activity. It is possible that in the crude extracts, in addition to chlorophyll, other factors such as endogenous antioxidants or inhibitors play an important role in the determination of lipoxygenase activity.

Since the specific activity of the lipoxygenase after purification in light- and dark-grown plants is comparable, and since the involvement of endogenous inhibitors or antioxidants was essentially ruled out (Table II), it can be concluded that in an in vitro system chlorophyll is an inhibitor. Therefore, chlorophyll could play an important role in the inhibition of lipoxygenase activity in light-grown leaves. These findings cast doubts on the assumption presented by Mohr (1972) that the increased activity of lipoxygenase in mustard seedlings grown under dark conditions represents synthesis of the enzyme. Mohr refers to the changes in activity when plants were changed from dark to light condition or vice versa as a demonstration of the rapidity of the threshold response in inhibition or resumption of lipoxygenase synthesis. Since all work was done with crude extracts, it is possible that the presence or absence of the chlorophyll in the extracts plays an important role in determining lipoxygenase activity, perhaps even to a greater extent than de novo synthesis of the enzyme.

Registry No. Lipoxygenase, 9029-60-1; chlorophyll *a*, 479-61-8; linoleate hydroperoxide, 78780-30-0.

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Enzymes of Glutamine and Asparagine Metabolism in Developing Wheat Grains

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Total protein, glutamate dehydrogenase, and aspartate pyruvate transaminase activities per grain increased during wheat grain development. Glutamate synthase and glutamate-pyruvate transaminase activities increased mainly during the early stages. Asparagine synthetase and glutamate-oxaloacetate transaminase activities declined at maturity. Activity of glutamine synthetase followed a decreasing pattern. Specific activities as well as activities per unit dry weight decreased at maturity. Compared to C-306 (low-protein variety), grains of Shera (high-protein variety) had higher activities of glutamate dehydrogenase, glutamate synthase, glutamate-pyruvate transaminase, and aspartate-pyruvate transaminase during later stages of grain development. It is suggested that in developing wheat grains, both the glutamate synthase cycle and glutamate dehydrogenase pathway may be operative in ammonia assimilation, the former predominating during early stages and the later playing a more active role during the later stages.

Protein malnutrition in developing countries is mainly due to the poor quality and quantity of protein in cereals, particularly wheat, which constitutes a major proportion of the staple diet. Any improvement in protein quality and quantity in cereals requires the basic understanding of the processes involved in protein accumulation in developing grains and a comparative study of these processes in a high- and a low-protein cultivar. The availability of amino acids at the site of protein synthesis in developing grain involves metabolism of amino acids and amides, particu-

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larly glutamine and asparagine, derived from leaves through the translocatory stream.

Before the discovery of glutamate synthase in bacteria (Tempest et al., 1970) and later in plants (Dougall, 1974; Lea and Miflin, 1974), ammonia was considered to be assimilated solely through the route involving glutamate dehydrogenase. However, the demonstration of glutamate synthase in plant tissues (Stewart and Rhodes, 1978; Lee and Stewart, 1978; Miflin and Lea, 1976) has changed the whole concept of ammonia assimilation and it is now generally agreed that ammonia is assimilated in roots as well as in shoots of higher plants via the glutamine synthetase–glutamate synthase pathway (Miflin and Lea, 1980), also called the glutamate synthase cycle (Rhodes et al., 1980). Glutamine synthetase and glutamate synthase have also been demonstrated in developing grains (Sodek and DaSilva, 1977; Misra et al., 1981), but it is still not known with certainty as to which pathway of ammonia assimilation is operative in these tissues. The developing grains are also known to transform extensively glutamine and asparagine, translocated from the leaves through the phloem, before being incorporated into storage proteins (Kolderup, 1980). The enzymes of glutamine and asparagine metabolism thus appear to play an important role in determining the extent of storage of protein accumulation in grains during development. Little work appears to have been done on this aspect, particularly in cereal grains. The present paper reports the activities of enzymes of glutamine and asparagine metabolism, including the enzymes of primary assimilation of ammonia in developing grains of low-protein and relatively high-protein wheat varieties.

MATERIALS AND METHODS

Plant Material. Wheat varieties, viz., C-306 (low protein, 9.8%) and Shera (high protein, 11.7%), were sown in pots in a greenhouse, under identical fertility conditions. The ears were tagged on the day of anthesis and harvested at weekly intervals, starting from 10 days after anthesis to 38 days. The harvested ears were kept under ice and the grains shelled.

Enzyme Extraction. Unless otherwise stated, all operations were carried out at 0–4 °C. Freshly shelled grains were homogenized in a mortar with a pestle by using ice-cold 0.1 M Tris-HCl buffer (pH 7.6). The homogenate was centrifuged at 10000*g* for 40 min in a refrigerated centrifuge. Clear supernatent was decanted and used as the enzyme extract for various assays. The extraction medium for asparagine synthetase was comprised of 0.2 M Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1 mM 2-mercaptoethanol (ME), and 0.15 M KCl. Addition of EDTA and ME to the extraction medium for enzymes other than asparagine synthetase did not increase the extractability of the enzymes.

Enzyme Assays. Glutamate dehydrogenase (Duke and Ham, 1976), glutamate synthase (Misra et al., 1981), glutamate-pyruvate transaminase (Segal and Matsuzawa, 1970), and aspartate-pyruvate transaminase activities were assayed by monitoring changes in absorbance at 340 nm, on oxidation of NADH to NAD^+. Assay for aspartate-pyruvate transaminase was based on that described for glutamate-pyruvate transaminase (Segal and Matsuzawa, 1970). The reaction mixtures for these enzymes consisted of the following: for glutamate dehydrogenase, 0.1 M Tris-HCl buffer (pH 7.6), 1.4 mL, 3 M NH_4Cl, 0.1 mL, 0.33 M α-ketoglutaic acid (neutral), 0.1 ML, 1 mM NADH, 0.2 mL, and enzyme extract, 0.2 mL; for glutamate synthase, 0.1 M Tris-HCl buffer (pH 7.6), 0.5 mL, 0.2 M glutamine, 0.2 mL, 0.2 M α-ketoglutaric acid (neutral), 0.2 mL, ME (1:100 v/v), 0.2 mL, 0.1 M KCl, 0.3 mL, 0.02 M EDTA, 0.2 mL, 1 mM NADH, 0.2 mL, and enzyme extract, 0.2 mL; for glutamate-pyruvate transaminase, 0.1 M Tris-HCl buffer (pH 7.6), 0.6 mL, 0.25 M α-ketoglutaric acid (neutral), 0.05 mL, 0.5 M alanine, 0.2 mL, lactate dehydrogenase, 2 units, 2 mM pyridoxal phosphate, 0.1 mL, 1.0 mM NADH, 0.2 mL, enzyme extract, 0.2 mL, and water to a final volume of 2.15 mL; for asparatate-pyruvate transaminase, 0.1 M Tris-HCl buffer (pH 7.6), 0.6 mL, 0.1 M oxaloacetic acid, 0.1 mL, 0.5 M alanine, 0.2 mL, 2 mM pyridoxal phosphate, 0.1 mL, lactate dehydrogenase, 2 units, 1 mM NADH, 0.2 mL, enzyme extract, 0.2 mL, and water to a final volume of 2.2 mL.

NADH was added as the last component. Incubation was carried out at 30 °C (25 °C in case of GDH). The activity in all the above cases has been expressed in terms of nmoles of NADH oxidized.

Glutamine Synthetase. Glutamine synthetase was assayed according to Rowe et al. (1970). The assay mixture (2.1 mL) consisted of 0.2 M imidazole–HCl buffer (pH 7.5), 0.4 mL, 0.1 M $MgCl_2$, 0.4 mL, ME (1:100 v/v), 0.4 mL, 0.5 M sodium glutamate, 0.2 mL, 0.5 M NH_2OH–HCl (freshly prepared by mixing equal volumes of 1 M NH_2 OH–HCl and 1 M NaOH), 0.2 mL, 0.1 M ATP, 0.2 mL, and enzyme extract, 0.3 mL. Incubation was carried out at 37 °C for 1 h.

Glutamate-Oxaloacetate Transaminase. Glutamate-oxaloacetate transaminase was assayed according to Wooten (1964). The reaction mixture contained 0.2 M phosphate buffer (pH 7.5), 0.2 mL, 0.02 M α-ketoglutaric acid (neutral), 0.1 mL, 0.5 M aspartic acid (neutral), 0.3 mL, and enzyme extract, 0.2 mL. The contents were incubated at 30 °C for 1 h in a shaking water bath.

Asparagine Synthetase. The asparagine synthetase assay was based on the method described by Rognes (1975). The reaction mixture in a final volume of 1.2 mL was comprised of 0.4 M aspartic acid (neutral), 0.2 mL, 0.1 M $MgCl_2$, 0.2 mL, 0.1 M ATP, 0.2 mL, 1 M NH_2OH–HCl (freshly prepared, neutral), 0.2 mL, ME (1:100 v/v), 0.2 mL, and enzyme extract, 0.2 mL. Incubation was carried out at 37 °C for 1 h in a shaking water bath. Reaction was terminated by addition of 2 mL of 5% $FeCl_3$ solution in 0.1 M HCl.

Soluble Protein. Soluble protein in enzyme extracts was estimated according to Lowry et al. (1951).

Total Protein. Total protein in the grains was determined by the micro-Kjeldahl method (Horwitz, 1975).

Each value in the tables is the mean of four independent estimations.

The methods followed for enzyme assays were standardized for substrate concentration, enzyme concentration, pH, and temperature of incubation.

RESULTS

Total Protein. Protein content per grain increased during development and was higher in Shera as compared to that in C-306 (Table I). When expressed as a percent of dry weight, it increased initially to a peak value and then decreased until maturity.

Glutamate Dehydrogenase (GDH). Glutamate dehydrogenase activity increased during grain development and was higher in Shera as compared to that in C-306, particularly at day 24 (Table II), where the activity in Shera was almost 2-fold higher. However, differences between the two varieties were considerably reduced when expressed in terms of specific activity.

Glutamine Synthetase (GS). Glutamine synthetase activity showed a decreasing trend during grain development (Table III). Differences between the two varieties

Table I. Total Protein in Developing Grains of Shera and C-306 Wheat

days after anthesis	total protein			
	mg/grain		% of dry wt	
	Shera	C-306	Shera	C-306
10	0.55 ± 0.03[a]	0.41 ± 0.02	13.78 ± 0.78	10.28 ± 0.82
17	1.81 ± 0.11	1.20 ± 0.09	15.45 ± 1.51	10.72 ± 0.72
24	3.66 ± 0.09	3.83 ± 0.26	13.85 ± 1.13	13.05 ± 0.99
31	5.05 ± 0.25	4.32 ± 0.31	12.54 ± 0.68	12.14 ± 0.85
38	8.28 ± 0.05	7.09 ± 0.06	11.66 ± 0.16	9.78 ± 0.25

[a] Each value in this and subsequent tables represents the mean of four independent estimations ± SE.

Table II. Glutamate Dehydrogenase and Glutamate Synthase Activities in Developing Grains of Shera and C-306 Wheat

days after anthesis	act., nmol of NADH/min				sp act., nmol of NADH min^{-1} $(mg\ of\ protein)^{-1}$	
	per grain		per mg of dry wt			
	Shera	C-306	Shera	C-306	Shera	C-306
			Glutamate Dehydrogenase			
10	9.63 ± 0.74	5.92 ± 0.37	1.78 ± 0.02	1.48 ± 0.05	23.33 ± 1.77	13.33 ± 0.74
17	34.07 ± 1.11	25.93 ± 2.44	2.92 ± 0.11	2.06 ± 0.16	27.77 ± 1.48	27.77 ± 1.11
24	64.07 ± 3.33	32.59 ± 1.11	2.43 ± 0.13	1.19 ± 0.08	29.63 ± 1.11	18.89 ± 0.99
31	100.74 ± 3.70	91.11 ± 1.85	2.50 ± 0.09	2.56 ± 0.05	65.92 ± 2.60	71.48 ± 2.96
38	113.33 ± 2.22	96.66 ± 0.74	1.60 ± 0.04	1.59 ± 0.02	32.96 ± 0.18	35.93 ± 1.11
			Glutamate Synthase			
10	3.70 ± 0.74	2.22 ± 0.00	0.86 ± 0.06	0.60 ± 0.06	11.11 ± 1.11	5.55 ± 0.04
17	25.93 ± 1.48	25.93 ± 1.48	2.23 ± 0.02	2.33 ± 0.15	21.11 ± 0.26	27.41 ± 0.74
24	32.22 ± 1.48	36.18 ± 1.85	1.22 ± 0.05	1.23 ± 0.07	14.81 ± 0.52	20.74 ± 1.41
31	33.33 ± 2.22	26.52 ± 1.85	0.83 ± 0.05	0.74 ± 0.03	11.11 ± 1.85	20.74 ± 1.48
38	33.70 ± 2.18	30.37 ± 3.00	0.53 ± 0.03	0.50 ± 0.03	10.74 ± 0.37	11.85 ± 1.11

Table III. Glutamine Synthetase and Asparagine Synthetase Activities in Developing Grains of Shera and C-306 Wheat

days after anthesis	act.				sp act., $(mg\ of\ protein)^{-1}$	
	per grain		per unit of dry wt			
	Shera	C-306	Shera	C-306	Shera	C-306
			Glutamine Synthetase (μmol of γ-Glutamylhydroxamate/h) per mg of dry wt			
10	19.33 ± 0.44	13.55 ± 0.28	4.29 ± 0.12	3.40 ± 0.08	56.28 ± 2.10	31.33 ± 1.05
17	16.44 ± 0.78	19.05 ± 0.89	1.40 ± 0.06	1.70 ± 0.08	13.39 ± 0.49	20.49 ± 3.08
24	12.94 ± 0.66	13.22 ± 0.22	0.49 ± 0.02	0.45 ± 0.01	5.94 ± 0.24	7.75 ± 0.24
31	7.16 ± 0.00	5.33 ± 0.16	0.18 ± 0.01	0.14 ± 0.00	4.72 ± 0.44	4.13 ± 0.27
38	7.55 ± 0.13	8.38 ± 0.33	0.11 ± 0.00	0.14 ± 0.01	2.28 ± 0.16	3.11 ± 0.17
			Asparagine Synthetase (ΔOD/h) per g of dry wt			
10	0.03 ± 0.00	0.06 ± 0.00	5.98 ± 0.60	1.57 ± 0.16	0.08 ± 0.01	0.01 ± 0.00
17	0.17 ± 0.01	0.09 ± 0.01	14.61 ± 1.02	7.88 ± 0.72	0.14 ± 0.00	0.98 ± 0.01
24	0.18 ± 0.01	0.22 ± 0.01	6.97 ± 0.24	7.62 ± 0.57	0.08 ± 0.00	0.13 ± 0.01
31	0.15 ± 0.00	0.16 ± 0.01	13.76 ± 0.21	5.62 ± 0.21	0.05 ± 0.01	0.11 ± 0.00
38	0.06 ± 0.00	0.03 ± 0.00	0.91 ± 0.12	0.58 ± 0.02	0.02 ± 0.00	0.01 ± 0.00

were not uniform. At day 10, when the activity per grain in Shera was at its peak, it had substantially higher activity than C-306. This was largely compensated for at day 17, when the activity in C-306 was relatively higher and reached its peak value.

Glutamate Synthase (GOGAT). Glutamate synthase activity per grain increased sharply from a very low value at day 10 after anthesis to a peak value at day 24 (Table II), whereafter it remained more or less constant in Shera. However, specific activity as well as activity per unit dry weight reached peak values at day 17, when Shera had slightly lower activity as compared to that of C-306.

Glutamate-Pyruvate Transaminase (GPT). Like GOGAT, glutamate-pyruvate transaminase activity recorded a sharp increase between days 10 and 17 after anthesis, and thereafter did not change substantially on a per grain basis (Table IV). However, activity per unit dry weight and specific activity decreased subsequently. The peak activity (day 17) per grain as well as per unit dry weight was higher in Shera as compared to C-306, although the specific activity was at the same level in the two varieties.

Glutamate-Oxaloacetate Transaminase (GOT). Glutamate-oxaloacetate transaminase activity per grain increased during development with a slight decline at the final stage (Table IV). On the other hand, activity per unit dry weight and specific activity decreased during grain development. At day 31, when the activity per grain was at its peak, Shera had considerably lower activity than C-306, irrespective of the manner of expression.

Asparagine Synthetase (AS). The activity of asparagine synthetase increased initially and later showed a decreasing trend (Table III). At day 17, Shera had much higher activity per grain as well as per unit dry weight than C-306.

Aspartate-Pyruvate Transaminase (APT). Aspartate-pyruvate transaminase activity per grain registered a sharp increase during development, although in C-306 the activity did not change much after day 24, resulting in considerably higher activity in Shera, as compared to

Table IV. Glutamate-Pyruvate Transaminase, Glutamate-Oxaloacetate Transaminase, and Aspartate-Pyruvate Transaminase Activities in Developing Grains of Shera and C-306 Wheat

days after anthesis	act. per grain		act. per mg of dry wt		sp act., (mg of protein)$^{-1}$	
	Shera	C-306	Shera	C-306	Shera	C-306
	Glutamate-Pyruvate Transaminase (nmol of NADH/min)					
10	4.44 ± 0.29	3.22 ± 0.00	1.04 ± 0.06	0.80 ± 0.08	13.70 ± 1.11	7.41 ± 0.52
17	40.74 ± 2.59	31.85 ± 2.96	3.49 ± 0.22	2.86 ± 0.19	32.96 ± 1.11	33.33 ± 2.96
24	29.62 ± 1.11	29.63 ± 0.37	1.12 ± 0.02	1.00 ± 0.02	14.07 ± 1.11	16.67 ± 0.52
31	35.92 ± 2.22	37.04 ± 2.22	0.89 ± 0.05	1.04 ± 0.06	23.33 ± 2.59	29.26 ± 2.22
38	48.52 ± 2.59	31.11 ± 3.33	0.68 ± 0.04	0.51 ± 0.03	14.07 ± 1.11	11.48 ± 0.37
	Glutamate-Oxaloacetate Transaminase (μmol of α-Ketoglutarate/h)					
10	1.09 ± 0.03	1.37 ± 0.08	242.45 ± 9.00	344.52 ± 7.00	3.16 ± 0.11	3.10 ± 0.20
17	2.63 ± 0.16	1.60 ± 0.17	224.99 ± 14.00	137.31 ± 2.90	2.15 ± 0.02	1.55 ± 0.14
24	1.84 ± 0.16	1.68 ± 0.13	69.36 ± 7.50	57.35 ± 4.50	0.87 ± 0.07	0.93 ± 0.09
31	2.63 ± 0.27	3.98 ± 0.13	75.61 ± 4.00	107.56 ± 10.00	1.90 ± 0.09	3.00 ± 0.14
38	2.20 ± 0.24	3.22 ± 0.13	63.58 ± 4.32	52.97 ± 2.10	1.32 ± 0.05	1.20 ± 0.03
	Aspartate-Pyruvate Transaminase (nmol of NADH/min)					
10	53.70 ± 1.48	98.15 ± 2.59	11.91 ± 0.35	24.51 ± 0.02	155.55 ± 2.58	225.18 ± 6.29
17	285.92 ± 7.40	228.52 ± 4.07	23.90 ± 0.67	20.41 ± 0.54	227.77 ± 4.07	240.74 ± 8.15
24	362.96 ± 13.70	443.70 ± 2.06	13.75 ± 0.58	15.08 ± 0.62	167.03 ± 17.77	254.44 ± 13.70
31	592.59 ± 14.44	424.07 ± 42.96	14.70 ± 0.36	11.91 ± 0.58	387.77 ± 21.48	336.29 ± 5.55
38	734.07 ± 16.66	461.48 ± 23.33	10.36 ± 0.23	7.39 ± 0.37	215.18 ± 4.92	172.59 ± 9.63

that of C-306, during the later stages (Table IV). The specific activity also increased up to day 31 and decreased thereafter. On the other hand, activity per unit dry weight in Shera increased initially up to day 17 with a subsequent decline during the later stages, whereas, in C-306, it decreased throughout development.

DISCUSSION

Although a barrier to the transport of nitrate from the phloem sap to the developing grain has been suggested in wheat (Nair and Abrol, 1977), and the presence of active nitrate reductase in this tissue has still not been demonstrated unequivocally (Nair and Abrol, 1973; Duffus and Rosie, 1978), it is now generally agreed that primary assimilation of ammonia into organic nitrogen does take place in the grain in situ (Duffus and Rosie, 1978). Demonstration of GDH, GS, and GOGAT (Miflin and Lea, 1976; Sodek and DaSilva, 1977; Storey and Beevers, 1978) activities in developing grains indicates that both the GDH pathway and GOGAT cycle may be operative in the primary assimilation of ammonia in this tissue. In animal tissues, GDH is primarily concerned with catabolic oxidative deamination of glutamate (Lehninger, 1975), and a similar situation is believed to exist in plants as well, possibly because of the fact that plant GDH has a very high K_m for ammonia (Chiu and Shargool, 1979). This argument, however, may not hold true, in the case of the ammonia being compartmentalized within the cell and concentrated at the site of GDH (Duffus and Rosie, 1978). Developing grains do not have catabolic activity to any appreciable extent, and the constantly increasing activity of GDH during development (Table II) strongly suggests that GDH might be actively associated with ammonia assimilation.

GDH activity follows a similar pattern as total protein in that both increase throughout grain development. GS activity, on the other hand, decreased during grain development (Table III), suggesting that this enzyme has probably little role to play in ammonia assimilation and subsequent protein accumulation. This is further evident from the failure to detect GS in endosperm (Garg, 1982) of either variety, which accounts for major proportion of the grain during development. GS appears to be located in the testa–pericarp, where it may be involved with the synthesis of glutamine, being subsequently incorporated into soluble enzyme proteins, particularly during early stages of grain development, when GS activity is at its peak.

Glutamine and asparagine translocated from the leaves into the developing grain may be transformed into glutamate and aspartate, major amino donors for transamination reactions, or may be used as such for protein synthesis. GOGAT shows a sharp initial increase in activity per grain, which remains more or less constant during the later stages. This enzyme, therefore, appears to be involved in transforming the translocated glutamine into glutamate without the involvement of glutamine synthetase, whose activity declines sharply during this period. Glutamate synthesized either through GDH by primary assimilation of ammonia or through GOGAT by transamidation of translocated glutamine may then be transaminated into other amino acids by GOT and GPT. AS activity increased initially and declined during the later stages, making its role doubtful in protein accumulation. The activity of APT, on the other hand, increased during development, suggesting that aspartate formed from oxaloacetate by APT may accumulate in the grain. Aspartate in the grain may also be obtained by deamination of asparagine either translocated from the leaves through phloem or formed within the grain by transamidation of glutamine by glutamine-dependent asparagine synthetase. It is, however, interesting to note that most of the enzyme activities, when expressed on a per unit dry weight basis or as specific activity, showed a decline during the later stages of grain development. Among other factors, this may be due to rapid accumulation of storage proteins and starch in the grain with concomitant decrease in soluble proteins.

Shera grains compared to C-306 had higher activities of GDH, GOGAT, GPT, and APT during the later stages of grain development, particularly between days 31 and 38 after anthesis, when the rate of protein accumulation in the grain was highest and the differences between the two varieties were maximum. On the other hand, GOT activity was much lower in Shera, whereas GS and AS activities were not very much different in the two varieties. It appears that higher activities of GDH and GOGAT in Shera may result in a higher concentration of glutamate, which may be either transaminated through GPT or immediately incorporated into storage proteins. The full significance

of changes in the activities of these enzymes may be known only after detailed analysis for free and protein amino acids, in vivo concentration of different metabolites, particularly in the vicinity of various enzymes, and chasing the fate of labeled NH_4^+, glutamine, and asparagine. On the basis of the results presented, it may, however, be suggested that in developing wheat grain, both the glutamate synthase cycle and the GDH pathway may be operative in ammonia assimilation, the former predominating during the early stages and the later playing a more active role during the later stages.

Registry No. GDH, 9001-46-1; GOGAT, 65589-88-0; GS, 9023-70-5; AS, 9023-69-2; GPT, 9000-86-6; GOT, 9000-97-9; APT, 9067-72-5.

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Peroxidative Activity of Oxyfluorfen with Regard to Carotenoids in *Scenedesmus*

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Autotrophic *Scenedesmus* cells were treated with difunon, a carotenogenesis inhibitor, thereby lowering the content of colored carotenoids by 50%. Photosynthetic electron transport remained effective to activate the *p*-nitrodiphenyl ether oxyfluorfen [2-chloro-4-(trifluoromethyl)phenyl 3-ethoxy-4-nitrophenyl ether) with subsequent peroxidation. Both carotenoid-deficient and normal cells exhibited the same peroxidative activity with oxyfluorofen. In both samples, peroxidative hydrocarbon formation was completely suppressed by an inhibitor of photosynthetic electron transport. We take this as evidence that in *Scenedesmus* and other green tissues light activation of *p*-nitrodiphenyl ethers essentially takes place through electron donation by the intact photosynthetic redox chain and not by direct interaction of diphenyl ethers with sensitized carotenoids.

Visible light is required for the phytotoxic activity of oxyfluorfen [2-chloro-4-(trifluoromethyl)phenyl 3-ethoxy-4-nitrophenyl ether; Kunert and Böger, 1981; Orr and Hess, 1981] as was previously described for other nitrodiphenyl ethers (Matsunaka, 1969a,b; Fadayomi and Warren, 1976). Peroxidations directed against membrane constituents apparently cause the essential phytotxic effects (Lambert et al., 1981; Orr and Hess, 1982). Peroxidative activity is decisively determined by substituents neighboring the *p*-nitro group (Lambert et al., 1983).

Obviously, a radical has to be originated by interaction of the *p*-nitrodiphenyl ether with a light-absorbing system. Right now there is no accordance as to how this may happen. Matsunaka originally proposed the essential role of (sensitized) carotenoids, since seedlings of white, carotenoid-free rice mutants exhibited tolerance, while yellow mutants were susceptible to *p*-nitrodipenyl ethers. Orr and Hess (1982), using etiolated, carotenoid-containing cucumber cotyledons, reported light-induced membrane leakage in the presence of peroxidizing diphenyl ethers. A decrease of colored carotenoids by a carotenogenesis inhibitor led to protection. Kunert and Böger (1981), however, treating the green microalga *Scenedesmus* with oxyfluorofen in the light, substantially prevented peroxidation and phytotoxicity by concurrent addition of diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea]. The latter herbicide blocks the photosynthetic electron flow, which was consequently postulated as being necessary to effectively initiate radical formation mediated by the *p*-nitrodiphenyl ether, giving rise to subsequent peroxidation of, e.g., polyunsaturated fatty acids (Sandmann and Böger, 1982a,b). Furthermore, peroxidation proceeded with red light (>610 nm), which did not activate carotenoids (Lambert et al., 1983a).

This paper resumes our studies with the alga *Scenedesmus*. Levels of colored carotenoids were decreased artifically by pregrowing cells in the presence of difunon [5-[(dimethylamino)methylene]-2-oxo-4-phenyl-2,5-di-

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