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METABOLISM OF PROTEIN, PEPTIDES AND AMINOACIDS IN AGEING ETIOLATED BARLEY LEAVES*

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Key Word Index—*Hordeum vulgare*; Gramineae; barley leaves; senescence; proteins; peptides; amino acids.

Abstract—Both light- and dark-grown primary leaves of barley show a reduction in protein after about 7 days. With this reduction in protein there is a rise in the concentration of low MW peptides (5–10 amino acid residues) reaching a peak at day 10. Total amino acids increased after day 7 showing a linear relationship with the diminishing amounts of protein. There was a redistribution of total amino acid within the ageing leaves. In the whole leaf there were considerable increases in most amino acids as the leaf aged, particularly glutamine, aspartate, histidine and glutamate. Glycine initially declined on illumination in 7-day-old leaves. The distribution of glycine, alanine and lysine within the ageing leaf was also examined. Labelling of low MW peptides enabled their metabolic origins (anabolic or catabolic) to be determined in ageing leaves kept in the dark and exposed to light

INTRODUCTION

Shortly after the start of germination, there is a rapid movement of amino acids, from the endosperm to the embryo, in cereals. In barley, this process reaches its maximum rate after 4 to 5 days [1]. It is accompanied by a 4 fold increase in peptidase activity between the 1st and 5th day of germination [2]. The transfer of nitrogen from the seed to the shoot is completed in about 8 days. The major translocated amino acids are glutamate and glutamine in young barley seedlings [3, 4], and with prolonged growth in the dark asparagine levels increase [5, 6]. Seedlings grown in the light utilise practically all the nitrogen in the grain for the synthesis of new protein [1, 7]. Barley seedlings grown in Vermiculite, in the dark, show fr. wt increases for at least 14 days [8] and may not even then be nitrogen deficient [9]. The following study gives an account of the changes which occur, with age, in protein, peptides and amino acids in etiolated barley leaves.

RESULTS AND DISCUSSION

Protein levels in ageing leaves

The amounts of total Tris–HCl soluble protein are shown in Fig. 1. In dark-grown leaves, protein reached a maximum of 8.36 mg/g fr. wt after 7 days. After this period there was a rapid decline to 3.7 mg/g fr. wt at day 11. Further reductions do not occur until after day 15 in dark-grown tissue. Seedlings, germinated in the dark for 16 hr, and then grown continually in the light, also show this loss of protein between day 7 and 11. When dark-grown seedlings are illuminated there is an increase in protein content. In 6-day-old dark-grown leaves this increase reaches a maximum after 48 hr light of 10.7 mg/g fr. wt. After 48 hr, the levels decline at a rate similar to that found in dark controls. In 8-day-old dark-grown material light effectively suppressed the loss of leaf protein keeping the levels at *ca* 8 mg/g fr. wt for at least 3 days. Fifteen day-old material, on illumination, still formed appreciable amounts of protein and reached 6.1 mg/g fr. wt after 3 days light.

It was previously shown that the oldest cells of the leaf (the tip) contained the greatest proportion of total leaf chlorophyll [8]. Preliminary experiments showed that the leaf tip also contained the largest proportion of total leaf protein [8]. An analysis was made of the protein content of the top 4 cm of the leaf at various ages (Table 1). After 8 days dark-growth, the 4 cm tips

Table 1. Soluble protein levels in the tips of ageing barley leaves

Age in dark (days)	Days in light	Protein (% total leaf protein)	Tip fr. wt (% total leaf fr. wt)
6	0	72.9	32.1
7	0	66.9	27.1
8	0	38.2	22.6
10	0	40.3	20.6
12	0	41.8	19.6
14	0	38.7	19.6
16	0	35.4	20.2
18	0	36.5	21.1
6	3	55.6	26.7
8	3	57.6	26.3
15	3	64.9	21

Seedlings were grown in the dark for up to 18 days. After 6, 8 and 15 days, plants were illuminated for a further 3 days. Primary leaves were harvested and the top 4 cm of the leaf removed. Protein and ft. wt were determined in the tips and in the remaining tissue.

* This paper is dedicated to Prof. E. W. Yemm (recently retired from the Department of Botany, University of Bristol) whose work on nitrogen metabolism in higher plants, particularly barley, is well known.

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maintained a constant proportion of 36–40% of the total leaf protein. This suggests that the decline in leaf protein (in absolute terms) is regulated at the same rate in the tops of the leaf as in the rest of the shoot, that is, protein turnover is at a constant rate throughout the leaf. The exception was in 6-day-old dark-grown leaves where the leaf tip contained no less than 70% of the total leaf protein with only 32.1% of the leaf mass. The leaf tips of 6- (and 7-) day-old dark-grown leaves are therefore particularly rich in protein and this feature disappears at day 8 as the leaves age.

After 3 days illumination (Table 1) the proportion of total leaf protein rose to 55.6% in 6-day-old dark grown leaves (against 42.3% in 9-day-old dark controls). In 8-day-old leaves, 3 days light increased tip protein to 57.6% (controls, 42.3%). In 15-day-old leaves light increased protein to 64.9% (controls 36.5%). Ageing leaf tips therefore show an increased capacity to form protein on illumination in relative, but not absolute, terms.

Amino acid levels in ageing leaves

Primary leaves, from seedlings grown in the dark, were analysed for total amino acids (Fig. 2). From a relatively low concentration after 6 days of growth, amino acids increase steadily to reach a peak at day 14, an increase of 76% in 8 days. This period of amino acid increase coincides with the period of decline in protein (Fig. 1), the two showing a good correlation up to day 14 (Fig. 3). After 14 days dark-growth there was a sharp reduction in amino acids. Since protein also declined in this period, this suggests that very old leaves increasingly utilise amino acids as respiratory substrates. When dark-grown leaves were illuminated, there was a reduction in amino acids of 16% in 6-day-old material. Older leaves (14 day) show an increase in amino acids after 24 hr light. As this was accompanied by a light-induced increase in protein, it appears that old leaves can undergo a rapid synthesis of amino acids. The reduction in amino acids in younger leaves, on illumination, probably reflects the utilisation of existing amino acid pools for protein production. The decline, after 24 hr light, in amino acids (16–19%) is similar to the increase in protein (21–26%).

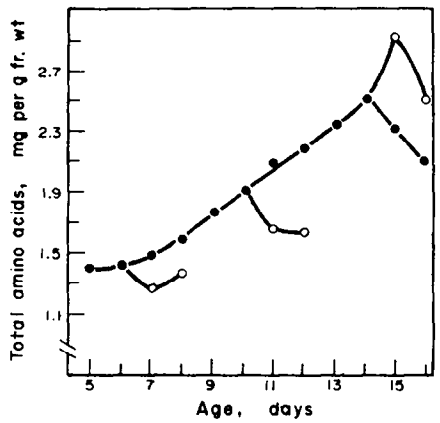

Fig. 2. Total amino acids in etiolated barley leaves. Seedlings were grown in the dark for up to 16 days. At intervals samples were illuminated for 24 and 48 hr. ●—●, dark grown; ○—○, transferred to the light.

These results however apply to the whole primary leaf and therefore mask changes of amino acid concentration which occur in different positions within the leaf. To investigate this possibility, seedlings were grown in the dark for up to 16 days; 6-, 10- and 14-day-old dark-grown plants being illuminated for 24 and 48 hr. The primary leaves were cut into 4 sections; the blade (expanded lamina) was cut into 3 equal lengths, the 4th section being the stem region enclosed by the coleoptile. The results (Fig. 4) show that 70% of the total leaf amino acid was present in the coleoptile region in 6-day-old dark-grown material. As the dark-grown leaf aged, the amino acids in this region declines to reach 20% of the total leaf amino acids at day 10. In the period 6–10 days the amino acid content of the blade sections increased from 10% section to about 25–30%. Since this redistribution is true in absolute as well as relative terms, it implies that there was an export of amino acids from the

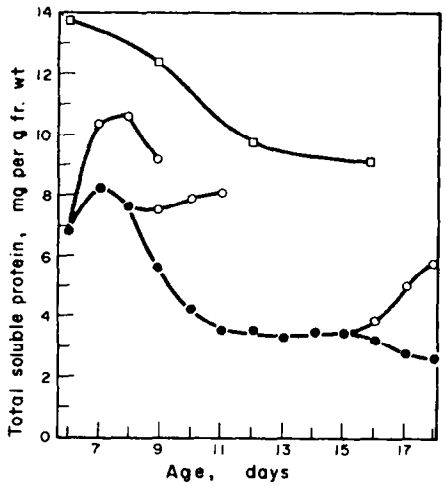

Fig. 1. Soluble protein in etiolated barley leaves. Seedlings were grown in the dark for up to 18 days. At intervals samples were illuminated for 24, 48 and 72 hr. Protein was extracted in Tris–HCl buffer (0.4 M, pH 7.5) and estimated by the method of ref. [20]. Plants were also illuminated for 16 hr after germination to provide protein levels for leaves under continuous light. ●—●, dark grown; ○—○, transferred to the light; □—□, continuous light.

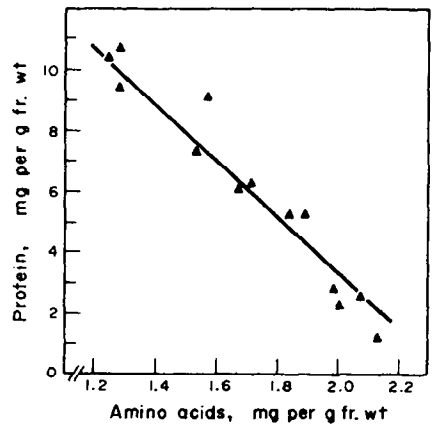

Fig. 3. Relationship between total amino acids and protein in etiolated barley leaves. Results were derived from Figs. 1 and 2 for seedlings grown in the dark for 6 to 14 days.

leaf base to the blade regions. The increases in the blade sections, however, were 65 to 86 μg/section in 4 days against a decline of 54 μg in the coleoptile region. The blade, therefore, appears to synthesise additional amounts of amino acids, though further quantities may have been derived from the roots. After 10 days in the dark, the top third of the leaf shows a reduction in amino acids from 85 μg to 50μg/section by day 12.

Individual amino acid levels in ageing leaves

Table 2 shows changes in 14 amino acids in ageing leaves. Amino acids not included occurred in amounts less than 0.15 μmol/g fr. wt. In the whole leaf, there were considerable increases in most amino acids as the leaf aged in the dark. Substantial increases were found between day 6 and 13 in glutamine (288%), aspartate (283%), histidine (86%), glutamate (50%), leucine (39%), threonine (37%) and iso-leucine (27%). The only amino acid to show any reduction over this period was glycine (21%). Compared to the whole sheet, the top 4 cm of the leaf showed quite different responses with age. No single amino acid showed any significant increase in the period 6–13 days in dark-grown material. Most amino acids reached a maximum by day 9 (day 10. Fig. 4) which declined thereafter. Exceptionally, asparagine declined rapidly throughout the period from day 6.

The relative concentration of each amino acid differs in the leaf tip compared to the whole shoot (Table 2). The concentration of glutamine in the 4 cm tip was 450–580% higher than that in the shoot as a whole. Aspartate and the basic amino acids, lysine, arginine and histidine, were also concentrated in the leaf tip. Again, asparagine was significantly different. In 6- and 9-day-old dark-grown leaves, the tips had 25 and 70% less asparagine when compared to the whole shoot. Asparagine disappeared from the leaf tip in 13 day-old tissue; this loss from the tip is short-lived. Electrophoretogram profiles showed a rapid rise in asparagine in the tip and in the lower

Table 2. Amino acids of the primary leaves of barley grown in the dark and exposed to light

Amino acid	Age in dark (days)	Amino acid (μmol/g fr. wt) Dark grown Whole leaf	Dark grown Top 4 cm	After 48 hr light Top 1 cm dark	After 48 hr light Top 1 cm light	% change on illumination
Asparagine	6	3.3	2.45			
	9	4.45	1.4	1.3	0	−100
	13	3.1	0			
Aspartate	6	0.6	1.38			
	9	1.4	2.18	0.80	0.5	−37
	13	2.3	1.3			
Glutamine	6	0.18	1			
	9	0.25	1.7	0.6	0.91	+52
	13	0.7	0			
Threonine	6	0.38	0.39			
	9	0.35	0.54	0.35	0.1	−71
	13	0.52	0.22			
Iso-leucine	6	0.26	0.29			
	9	0.34	0.32	0.13	0.03	−77
	13	0.33	0.13			
Leucine	6	0.18	0.17			
	9	0.25	0.32	0.2	0.04	−80
	13	0.25	0.11			
γ-Amino butyrate	6	0.21	0.07			
	9	0.24	0.33	0.29	0.07	−76
	13	0.17	0.14			
α-Alanine	6	0.51	0.57			
	9	0.8	0.88	0.68	0.6	−12
	13	0.64	0.44			
Glycine	6	0.33	0.29			
	9	0.29	0.34	0.26	0.19	−27
	13	0.26	0.14			
Histidine	6	0.36	0.45			
	9	0.58	0.01	0.75	0.16	−79
	13	0.67	0.24			
Arginine	6	0.13	0.23			
	9	0.22	0.25	0.18	0.07	−61
	13	0.17	0.05			
Lysine	6	0.34	0.46			
	9	0.49	0.7	0.55	0.29	−47
	13	0.52	0.45			
Glutamate	6	0.6	0.71			
	9	1	1.3	1.1	1.08	−2
	13	0.9	0.8			

The amounts of all other amino acids were less than 0.15 μmol/g fr. wt. Seedlings were grown in the dark for 6 to 13 days. Primary leaves were stripped from the second leaf, cut at the mesocotyl or cut 4 cm below the leaf tip. Nine-day-old dark-grown seedlings were illuminated for 48 hr. The top 1 cm of the leaf was removed together with the same region from 11-day-old dark-grown controls.

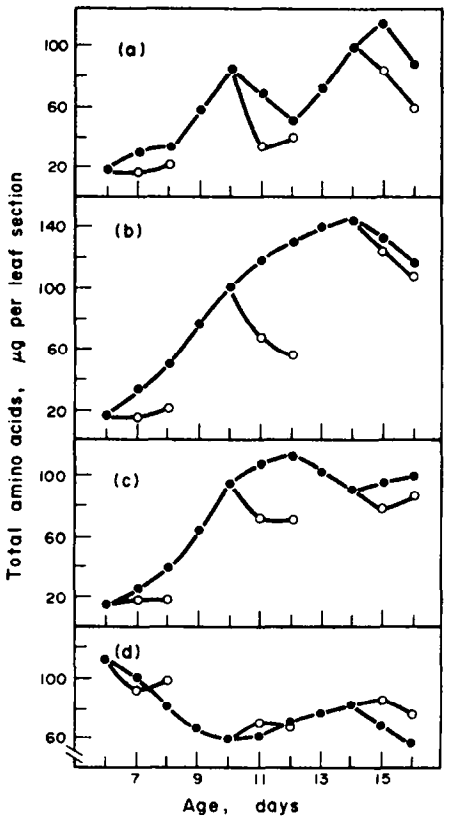

Fig. 4. Total amino acid distribution in etiolated barley leaves. Seedlings were grown in the dark for up to 16 days. At various ages samples were illuminated for 24 and 48 hr. Leaves were cut into 4 sections; the blade was divided into 3 equal lengths, the stem region enclosed by the coleoptile formed the 4th region. (a) top, (b) middle, (c) bottom and (d) coleoptile region. ●—●, dark grown; ○—○, transferred to light.

parts of the leaf, after 14 or 15 days of dark growth. This is in agreement with other reports of increased asparagine in etiolated leaves [5].

To quantify the effect of light on amino acid production, 7-day-old dark-grown seedlings were illuminated for 48 hr. The top 1 cm of the leaf tip was analysed, together with those from 9-day-old dark-grown controls. Light induced a reduction in most amino acids (Table 2) but most noticeably in asparagine, the leucines and in the basic amino acids. Glutamate showed little change in illuminated tissue whilst glutamine increased by some 52%. In very old leaves (16–18 day dark-grown) light treatment resulted in the appearance of considerable quantities of asparagine both in the tips and basal parts of the shoot. The more 'moribund-looking' leaves contained the largest amount of asparagine which in some cases comprised 60% of the total leaf amino acids. Substantial increases were also observed for histidine, arginine and lysine. The amino acid increases observed in 14-day-old leaves on illumination (Fig. 4) were largely in asparagine.

Glycine, alanine and lysine levels in ageing leaves

Three amino acids were chosen for further investigation. Glycine was selected because it was the only amino acid to show a rapid depletion in dark-grown leaves after day 6. Alanine was studied as a comparison and lysine because it appeared to behave differently to glycine and because of its apparent role in inhibiting chlorophyll and protein degradation in the dark [11].

A detailed analysis of these amino acids in ageing leaves is shown in Fig. 5. The changes in glycine up to day 10 have previously been discussed [12], particularly in relation to porphyrin synthesis. After day 10, with the onset of senescence, there was a steady rise in glycine to reach 4.2% of the total amino acids at day 14. Alanine and lysine also increased in this period.

Little increase was observed in any of the 3 amino acids between day 7 and 10 of dark-growth. This period, however, had the maximum rate of decline in protein level (Fig. 1). Although the products of proteolysis may be deaminated very rapidly, it appears more likely that a substantial proportion of proteolytic products are held as peptides during this period. This was confirmed [8] by the appearance of numerous small bands of peptides in electrophoretograms. Since these peptides did not incorporate ^{14}C labelled CO_2, acetate, glycine or glutamate, they were considered to be protein breakdown products. At, or shortly after, day 10, these peptides are quantitatively reduced and virtually disappear in 12-day-old dark-grown material. It appears that these peptides are finally broken down to their constitutent amino acids only after day 10, and this coincides with the rapid rise in most amino acids between days 11 and 14. Illumination induced a rapid reduction in glycine in 6-day-old dark-grown tissue depleting the pools by 42% in 24 hr.

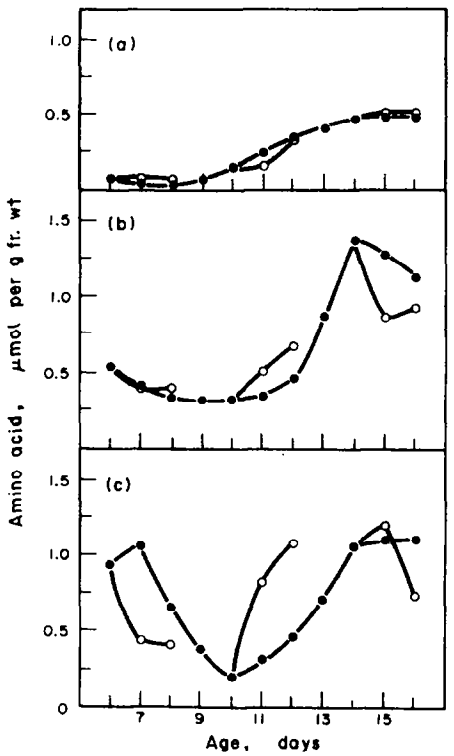

Fig. 5. Glycine, alanine and lysine in etiolated barley leaves. Seedlings were grown in the dark for up to 16 days. After 6, 10 and 14 days, samples were illuminated for 24 and 48 hr. (a) lysine, (b) alanine, (c) glycine. ●—●, dark grown; ○—○, transferred to the light.

No such decrease was observed for alanine or lysine. After 10 days dark growth illumination increased glycine and alanine.

In order to localise the changes in the 3 amino acids, the leaf blade of dark-grown and illuminated material, was cut, as before, into 3 equal lengths with the coleoptile enclosed stem forming the 4th section. The distribution of amino acids in each section is given in Figs. 6, 7 and 8.

Unlike the situation for total amino acids (Fig. 4) the distribution of glycine (Fig. 6), throughout the leaf, shows little change with age. In all parts of the shoot, glycine is at its highest in 6 and 7 day old material and a second maximum occurs in the blade sections at or after 16 days. The coleoptile region shows a substantial decline after 14 days and a characteristic reduction also occurs between day 7 and 11 throughout the shoot. The rapid increase on illumination in 10-day-old leaves also occurred throughout the leaf and again was most pronounced in the upper parts of the leaf blade. The decline on illumination of 14-day-old leaves occurred, initially, only in the coleoptile region, the blade sections responding in the same way only after 24 hr light.

The small decrease in alanine in the young shoot (Fig. 5) masks the much greater decline in blade sections (Fig. 7). There was a sharp reduction between day 6 and 10 in the top and middle sections of the dark-grown leaf, accompanied by a small increase in the coleoptile region. These

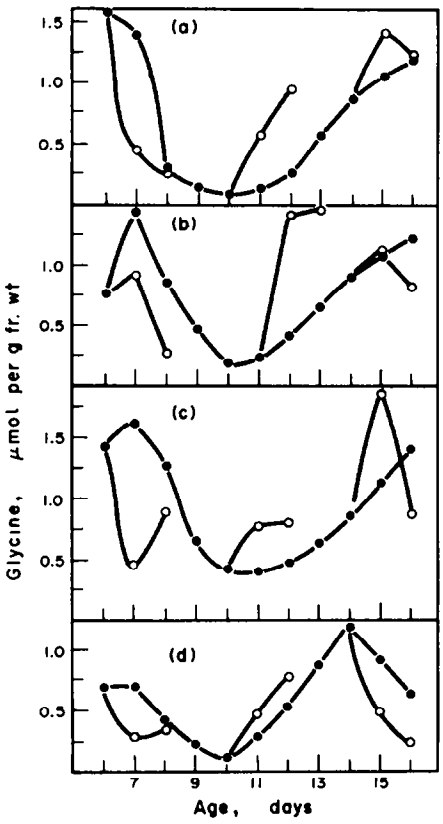

Fig. 6 Glycine distribution in etiolated barley leaves. Seedlings were grown in the dark for up to 16 days and at various ages, illuminated for 24 and 48 hr. The primary leaves were cut into 4 sections: the stem region enclosed by the coleoptile formed the 4th section. (a) top (b) middle (c) bottom and (d) coleoptile region ●—●, dark grown; O—O, transferred to the light.

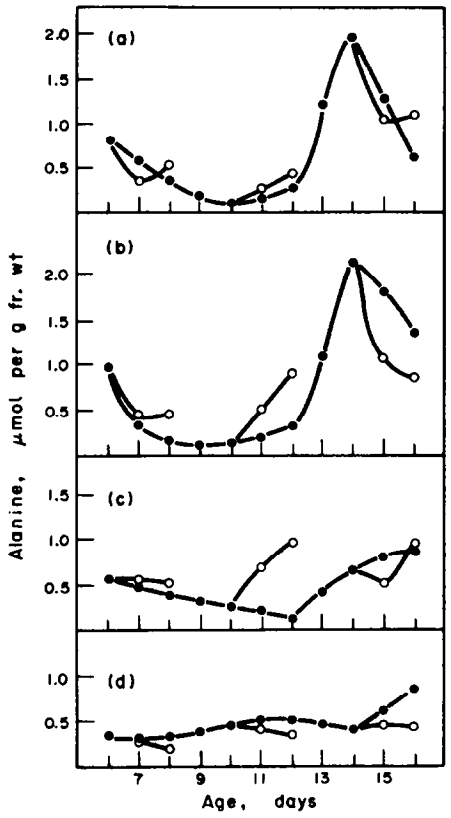

Fig. 7. Alanine distribution in etiolated barley leaves. (a) top (b) middle (c) bottom and (d) coleoptile region. ●—●, dark grown; O—O, transferred to light.

reductions start one or more days before the decline in glycine. The increase which occurs in older leaves was delayed until the 12th day in the case of alanine; one or two days later than that observed for glycine. The 5 fold increase in alanine between day 12 and 14 was so characteristic that it was possible to 'date' electrophoretogram profiles merely by observing the size of the alanine band. Equally pronounced was the rapid reduction in the top and middle sections of the blade after day 14. Light treatment induced relatively small reductions in 6 and 14 day-old material. Again 10-day-old dark-grown leaves showed an increase after illumination. Unlike glycine, however, the light induced increase was confined to the middle and bottom $\frac{1}{3}$ of the leaf.

Lysine concentrations were low in young barley leaves forming less than 0.5% of the total amino acids. The small amount present in 6 to 8 day-old dark-grown leaves was distributed more or less equally, throughout the leaf sections. There was no reduction after day 7 as was found for glycine. Rather, there was a steady increase from about the 8th or 9th day of dark-growth, continuing to at least day 16. Light had little effect in 6 and 10 day-old dark-grown leaves. There was, however, a sharp reduction in the top and middle sections of the blade in 16-day-old plants after 24 hr light. This reduction coincides with an increase in the bottom $\frac{1}{3}$ of the blade and coleoptile region.

Peptides in ageing leaves

Electrophoretic separations of barley extracts showed

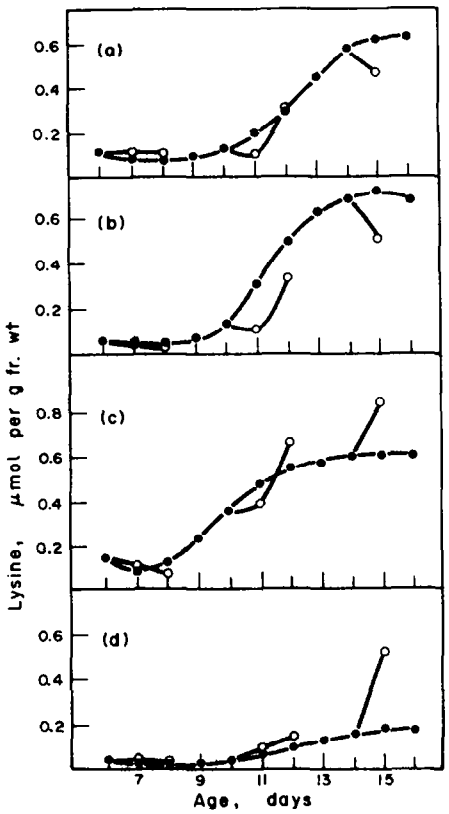

Fig. 8. Lysine distribution in etiolated barley leaves. (a) top (b) middle (c) bottom and (d) coleoptile region ●—●, dark grown; O—O, transferred to light.

the appearance of ninhydrin-collidine-staining bands of low MW peptides, an observation in agreement with those of Cocking and Yemm [13] who used ion-exchange techniques. Some, but not all, of these peptides became radioactive after glycine-[^{14}C] feeding for 2 hr before extraction. The peptides were authenticated by hydrolysis revealing up to 10 amino acids. Labelled peptides after hydrolysis gave up to 5 ^{14}C-amino acids viz. glycine, serine, alanine, aspartate and glutamate. The mobility of the peptide bands on electrophoretograms coincide with those of a wide range of low MW (500–1500) peptides.

These peptides may appear as a result of peptide synthesis from amino acids (aminoacyl adenylates) destined for protein synthesis (anabolism). Such peptides become labelled when glycine-[^{14}C] (and its derivatives) become incorporated. Peptides may also arise from protein and polypeptide degradation (catabolism). Such peptides were unlikely to become radioactive in short (2 hr) feeding experiments. Therefore by estimating the number of radioactive and non-radioactive peptides it is possible to determine their metabolic origins.

Seven-, 10- and 14 day-old dark-grown leaves were cut into 2 cm segments, the segments being derived from the 2nd to 4th cm below the primary leaf tip. After 2 hr incubation in the dark in phosphate buffer (10^{-2}M, pH 7.2) containing glycine-[^{14}C] (2.5μCi/50 segments) peptides were extracted, purified and estimated as given in the Experimental. To demonstrate the effect of light on peptide formation leaves were grown in the dark for 6, 9 and 13 days before illumination for 24 hr.

Table 3. Low MW peptides in dark-grown barley leaves

M_{gly} band	Age (days) 7	10	14
	Score	Score	Score
0.25–0.27	—*	1*	1
0.29–0.33	1	3	2
0.34–0.37	—†	1*	1*
0.38–0.41	1	3	2
0.43–0.46	—*	1	—
0.47–0.48	1	1	—
Total score	3	10	6
Labelled score	0	2	1
Unlabelled score	3	8	5

Seedlings were grown in the dark for 7, 10 and 14 days. Primary leaves were cut and a segment 2 cm long removed from the leaf 2 cm below the tip. Segments were incubated in 2.5 μCi glycine [U-^{14}C] (110–114 mCi mmol) in phosphate buffer, pH 7.15. After 2 hr in the dark the segments were washed and analysed for peptides. Stained peptide bands after electrophoresis and staining were quantified on a score of 1 = faint, 2 = small and 3 = large. Radioactivity is expressed as * = 3–9% of total DPM uptake and † = 10–18% of total DPM uptake. M^{gly} refers to mobility relative to glycine.

The results indicate that most of the peptides formed in dark-grown leaves were derived from catabolism (Table 3). The 10-day-old dark-grown leaves reached a high score of 8, declining to 5 in 14-day-old material. These high scores occur 3 or more days after the onset of the reduction in protein (Fig. 1) suggesting that the degradation of protein to low MW peptides is a slow process in 7- to 10-day-old leaves. Peptide synthesis (anabolism) also occurs in the dark. However, none of the labelled peptides in 7-day-old leaves were visible when stained, suggesting that the pools of labelled peptides were small and perhaps rapidly turned over. In older leaves, the labelled peptides did stain, either as a result of increased rates of peptide synthesis and (or) slower incorporation into polypeptides and protein. Band M^{gly} 0.34–0.37 was consistently radioactive at all ages. Bands M^{gly} 0.25–0.27 and 0.43–0.46 were radioactive only in younger tissues suggesting that the same 'type' of peptide is formed, anabolically, in the dark at all ages, but the amount declines (or is more rapidly turned over) in older leaves.

When etiolated leaves were illuminated, the most obvious feature was an increase in the number of peptides (Table 4). In 7-day-old leaves, after 24 hr light, 6 peptides were present (as against 3 in dark controls). The total score of 9 in young leaves in the light, gave 4 for anabolism and 5 for catabolism, suggesting that light induced an increase in both forms of peptide metabolism. In older leaves (10 and 14 days), after 24 hr light, the unlabelled (catabolic) score declined compared to dark controls. This decline, however, coincides with an increase in free amino acid in some or all parts of the leaf (Fig. 4). It would appear that light need not necessarily suppress the catabolic formation of peptides but rather increases the rate of hydrolysis to free amino acids. This may be to ensure an adequate supply of substrates during the establishment of an autotrophic state. The reverse is true in 7-day-old leaves where light increased the score for peptide catabolism without increasing the amounts of free amino acids (Fig. 4). This indicates that in young

Table 4. Low MW peptides in dark-grown barley leaves after 24 hr light

M^{gly} band	Age (days) 7	10	14
	Score	Score	Score
0.25–0.27	1	1	—
0.30–0.32	2*	2*	1*
0.33–0.34	—	1	—
0.35–0.37	2	1	1†
0.38–0.41	2*	1*	1
0.42–0.45	1	1	1
0.47–0.48	1	1	—
0.48–0.5	—†	—†	—†
Total score	9	8	4
Labelled score	4	3	2
Unlabelled score	5	5	2

Seedlings were grown for various times then illuminated for 24 hr before cutting. Isotope feeding was in the light.

leaves only, protein turnover is stimulated in the light, resulting in an increased number of low MW peptides from protein breakdown and that these peptides are not further hydrolysed until 2 or 3 days later. This 'hold-over' of peptides appears to operate in the dark in 7- to 10-day-old leaves as well. It would explain the appearance of the "considerable quantities of bound amino acids, probably contained in peptides", reported by Yemm [13] in 10-day-old barley.

The labelling of peptides obtained in the light is quite different from that in the dark. A peptide at M^{gly} 0.48–0.5 was invariably radioactive in illuminated leaf extracts at all ages, and only occasionally detectable in dark-grown material. Similarly, labelling of 3 peptides in dark-grown leaves, ceased on illumination. Light, therefore induced a change in type as well as in the amount of peptide synthesised. The major protein of higher plants is fraction 1 protein[14] which is synthesised rapidly in barley leaves on illumination[15]. It is tempting to infer from the light induced changes in labelling that one or more of these peptides was destined for fraction 1 protein. The obvious candidate would be M^{gly} 0.48–0.5. On hydrolysis this was found to contain glycine, serine and aspartate in the ratio of 4:2:1. A fourth component thought to be an S-amino acid was also present. In numerous experiments this peptide contained some 13–15% of the total isotope after glycine-[^{14}C] feeding suggesting (since its was a minor peptide) that it is rapidly turned over in illuminated leaves.

GENERAL DISCUSSION

There appears to be a strict sequence for the changes in the concentrations of protein, peptide and amino acids which occur in ageing barley leaves. Day 0–6: a period of rapid protein formation within the primary leaf, particularly in the leaf tip, during which time the amounts of amino acids remain relatively low. Day 7: protein reaches its maximum; peptide synthesis from amino acids continues in both dark and illuminated leaves, with rapid incorporation into protein. Day 8–10: a period of rapid reduction in protein with an increasing formation of peptides derived from proteolysis; peptide synthesis from amino acids continues, however, but at a reduced rate both in illuminated and dark-grown leaves. Day 11–14: relatively constant amounts of protein: peptides derived from catabolism decline, probably as a result of their rapid hydrolysis to amino acids; illumination during this period increases amino acids perhaps because of an even faster rate of peptide hydrolysis. Day 15–18: a second period of reduction in protein but unlike the first (8–10 days) it is accompanied by a substantial reduction in total amino acids.

There was no indication that the ability to form protein on illumination was seriously impaired in old leaves as was found for chlorophyll [10]. Thomas and Stoddart [16] working with a meadow fescue mutant, conclude that the loss of chlorophyll from detached leaves was not directly related to a decline in protein. It would appear that in barley also, the loss of the ability to form chlorophyll is not due to the loss of the ability to form protein as such, although the formation of particular enzymes, notably those synthesising δ-aminolevulinic acid, are seriously affected [17].

While the concentrations of protein are maintained at relatively constant proportions in ageing leaves those of amino acids change considerably. Some of these changes appear to involve translocation, the direction altering with age. Up to day 10 there appears to be an acropetal movement after which the flow is reversed. The bottom ⅓ of the older leaves appears to act as a sink, perhaps drawing free amino acids from the upper parts. The bottom ⅓ of the blade contains the nodes from which the 2nd and 3rd leaves arise. There is then, a controlled basipetal movement of amino acids from the primary leaf towards the 2nd and 3rd leaves in the ageing barley seedlings.

Glutamate, glutamine and aspartate appear to be the principal amino acids involved in translocation in young leaves, increasing considerably in the period 6–9 days. Asparagine and the 3 basic amino acids, lysine, arginine and histidine become the dominant amino acids in old leaves.

It would appear that the degradative role suggested for serine [18, 19] and the protective role of lysine [11] in controlling senescence noted in exogenous feeding experiments may also have relevance for intact leaves.

EXPERIMENTAL

Barley seeds (*Hordeum vulgare*, cv Proctor) were imbibed in H_2O for 16 hr, planted in trays of Vermiculite and germinated in the dark at 26°. Light intensity was 3500 lx at the level of the seedlings.

Total protein and amino acids. Protein was extracted in 0.4 M Tris–HCl, pH 7.5 and determined by the method of ref. [20]. Amino acids were extracted with EtOH at 70° and after reducing to a small vol. lipids were removed by extraction with Et_2O. Total amino acids were determined using a sensitised ninhydrin method [13].

For automated amino acid analyses leaves (250–300 mg fr. wt) were ground in liquid N_2 and exhaustively extracted with MeOH–$CHCl_3$–H_2O (12:5:3). The bulked extracts were partitioned in $CHCl_3$–H_2O (1:1) and the aq. phase reduced in vol. *in vacuo*. Half of each sample was incubated for 18 hr in glutaminase and asparaginase [12] and the amino acids determined with an autoanalyser. Alcoholic amino acid extracts were also analysed by high voltage zone paper electrophoresis (formate–acetate electrolyte, pH 1.95 at 85V cm for 28 min).

Low MW peptides (5–10 amino acid residues) were extracted in aq. EtOH and separated by electrophoresis as for amino

acids. For hydrolysis, peptides were eluted in 1% HOAc into glass vials and evapd to dryness. 50% HCl (0.2 ml) was added before sealing and heating at 100° for 24 hr. Hydrolysed samples were diluted with H_2O and the amino acids analysed by electrophoresis. Dried electrophoretograms were cut longitudinally, half being stained with ninydrin–collidine, the other half being subjected to autoradiography for up to 6 weeks. Stained bands were quantified on a score scale of faint 1, small 2, and large 3. Peptides were identified from their chromatographic mobility relative to glycine (M^{gly}). Radioactivity was measured by liquid scintillation with a full quench correction.

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