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AMINO ACIDS AND QUATERNARY NITROGEN COMPOUNDS IN THE GERMINATING WHEAT GRAIN

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Key Word Index—*Triticum*; Gramineae; wheat; amino acids; quaternary nitrogen compounds; glycine betaine; choline; germination; gibberellic acid; aleurone tissue.

Abstract—The tissues of the quiescent wheat grain contained free amino acids and quaternary nitrogen compounds. During germination the amino acid levels increased several fold. In the aleurone tissue and starchy endosperm glutamine was the predominant amino acid. Asparagine was predominant in the seedling tissues. Choline and glycine betaine were the principal quaternary nitrogen compounds present. The aleurone tissue and the embryo/seedling contained large quantities of glycine betaine. The increase in free amino acid levels in the aleurone tissue during the first 2 days of germination occurred independently of the embryo. After the second day, the further increase in levels was dependent upon the presence of the embryo and of gibberellic acid (GA). Estimation of the individual amino acids and quaternary nitrogen compounds released from incubating aleurone layers into aqueous media revealed a selective release of some compounds and retention of others. The process was regulated by GA. Possible mechanisms for the release of amino acid and its control by GA are discussed.

INTRODUCTION

The aleurone tissue of cereal grain forms and secretes hydrolytic enzymes in response to gibberellic acid (GA) [1]. Some of these enzymes, and in particular α-amylase and protease, are synthesized *de novo* from amino acids which, in turn, are derived from the hydrolysis of the reserve protein of the tissue [1]. Density labelling experiments using ^{18}O-water to label the amino acids derived by hydrolysis have, in fact, shown that most of the α-amylase and protease arise in this way [2, 3]. Some other proteins which increase in amount during germination, but not necessarily as the result of GA-action, may be similarly synthesized *de novo* from amino acids; enzymes of the tricarboxylic acid cycle and the glycolytic pathway [4] are probable examples.

There is now evidence that GA acts in the aleurone cell to induce the synthesis of polydisperse, poly(A)-RNA (presumed to be a family of *m*RNAs) [5–7], and consequently it has been proposed that the hormone acts principally at the transcriptional level of genetic control. Although this concept may adequately explain the induction of protein synthesis during germination, it does not necessarily clarify the situation regarding the hydrolysis of the reserve protein which, is, of course, a prerequisite for the synthesis of the new protein. The control of protein hydrolysis and of the levels of the resulting amino acids in aleurone tissue has not, in fact, been studied in any detail. Indeed, other than in the studies of Folkes and Yemm [8] and Margaris and Thanos [9], few detailed studies of amino acid levels in germinating cereals have been made. In the present investigation we have repeated and extended some of their experiments to obtain definitive data on the levels of this group of compounds in germinating wheat and we have sought to characterise some of the control systems which operate. In addition we have obtained data for two related quaternary nitrogen compounds, choline and glycine betaine, which are known to be present in germinating wheat [10].

RESULTS

Total tissue levels of amino acids and quaternary nitrogen compounds

The total free amino acids in the aleurone layer and starchy endosperm increased in quantity without interruption from the onset of germination up to the 5th or 6th day of germination (Figs. 1a, b). Similarly in the embryo the levels increased from the onset of germination, and after the second day the rate of increase was dramatic so that by the 6th day the levels were much higher than in either the aleurone layer or starchy endosperm (Fig. 1c).

The compositions of the free amino acid fractions from ungerminated grain and grain germinated for 4 days are presented in Table 1. These data showed that glutamine was the predominant amino acid in the aleurone layer and starchy endosperm in both the ungerminated and the germinated grain. Although proline was present in relatively small amounts in the ungerminated grain it increased to levels approaching those of glutamine by the 4th day. Changes in the proportions of the other amino acids were less marked. Glutamine, along with asparagine, alanine and proline, was also a major amino acid in the embryo of the ungerminated grain. In contrast to the situation in the aleurone layer and starchy endosperm, there was a large increase in the proportion of asparagine in the embryo during germination so that by the 4th day it accounted for 45% of the total free amino acids in that tissue. By the 6th day there was a further

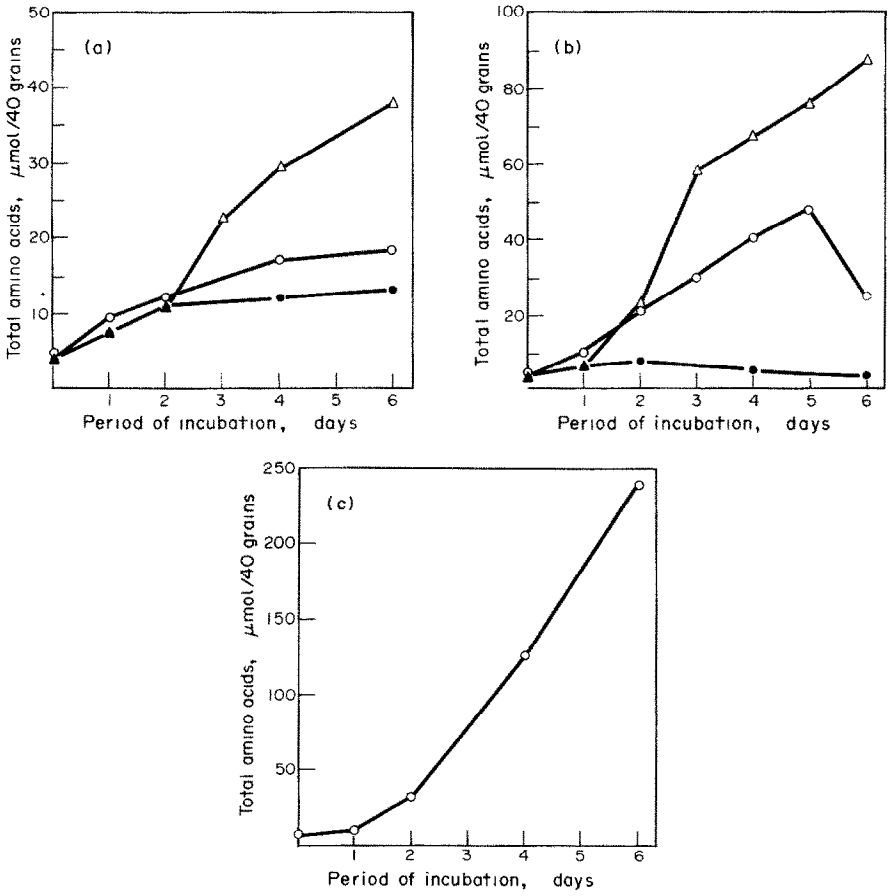

Fig. 1. Total free amino acid levels in (a) the aleurone tissue, (b) the starchy endosperm and (c) the embryo tissues of wheat. ○, levels in germinated grain; ●, levels in incubated endosperms; △, levels in endosperms incubated with 1 μm GA

Table 1. Amino acids and quaternary nitrogen compounds in the tissues of ungerminated wheat grain and grain germinated for 4 days (μmol/40 grain)

	Aleurone layer		Starchy endosperm		Embryo	
	Ungerminated	Germinated	Ungerminated	Germinated	Ungerminated	Germinated
Aspartate	0 18	1.20	0.33	2.24	0 23	3.87
Asparagine	0.52	0.72	0.70	0.86	0.85	56.5
Threonine	0.12	0.75	0.20	1.57	0.21	4.25
Serine	0.33	1.34	0.34	2.99	0.21	4.40
Glutamate	0.11	1.64	0.15	1.24	0.16	5.74
Glutamine	1.23	4.76	2.52	13.4	1.16	12.4
Proline	0.16	4.24	0.22	6.70	0.59	6.11
Glycine	0.12	0.29	0.22	0.62	0.20	0 58
Alanine	0.39	1.91	0.59	2.20	0.71	5.13
Cysteine	nd	0.17	0.03	0.32	0.02	nd
Valine	0.18	1 78	0.63	3.35	0.19	5.44
Methionine	0.03	0.39	nd	0.55	nd	0 42
Isoleucine	0.09	1.28	0.14	2.60	0.07	4.27
Leucine	0.21	1.98	0.42	4.27	0.17	3.91
Tyrosine	0 05	0.82	0.38	1.88	0.21	0.98
Phenylalanine	0.11	1.76	0.58	3.77	0.27	2.49
Histidine	0.13	0.73	0.47	1.58	0.37	3.25
Tryptophan	1.04	2.04	0.37	1.36	nd	1.10
Lysine	0.05	0.30	0.17	1.07	0.18	0.90
Arginine	0.09	0.65	0.32	1.30	0.40	0.99
Total	5.14	28.8	8 78	53.9	6 20	122.7
Choline	0.43	1.52	0.37	0.68	0.26	0.97
Glycine betaine	8.83	5.96	0.88	1.58	5.39	13.5

Table 2. Effects of inhibitors of protein synthesis on the GA-induction of increased amino acid levels in incubated endosperms. The endosperms were incubated for 4 days in the various media and then analysed. Values for α-amylase are presented for comparison

Incubation medium	Amino acids (μmol/40 grain) Aleurone layer	Starchy endosperms	α-amylase activity (S.I.C. units/40 grain) Aleurone layer + starchy endosperm
Water (control)	11.1	8.1	0
1 μM GA	23.8	61.9	110
1 μM GA + cycloheximide (3 μg/ml)	11.4 (95)	13.1 (91)	3 (97)
1 μM GA + mikamycin (50 μg/ml)	16.3 (59)	36.9 (28)	23 (79)

The figures in parenthesis refer to the percentage inhibition of the GA-stimulated level.

increase so that it accounted for more than 60% of the total.

A preliminary analysis of the quaternary nitrogen compounds identified choline and glycine betaine as the predominant components of this fraction. Glycine betaine was by far the most abundant of the two, and it was present in large amounts. The aleurone layer and embryo of the ungerminated grain, for example, contained as much glycine betaine as total free amino acids (Table 1). The changes in glycine betaine contents of the various tissues during germination were, however, different from those of the total free amino acids. Thus, the amounts of glycine betaine in the embryo and the starchy endosperm increased only by about two-fold up to the 4th day, and the level in the aleurone layer actually decreased. Choline, on the other hand, increased three-fold in the aleurone layer and about four-fold in the embryo; i.e. the choline changes were similar to those of the amino acids in those tissues. Choline levels in the starchy endosperm increased by less than two-fold.

The role of the embryo and of gibberellins in controlling the levels of free amino acids in the reserve tissues was clearly revealed by experiments using de-embryoed grains (endosperms). These experiments showed that the two-fold increase in the total free amino acid level occurring in the aleurone layer during the first 2 days was not dependent upon the presence of the embryo or any hormonal factor such as GA emanating from the embryo (Fig. 1a). The small upward displacement of the whole grain values compared with those of the endosperms during this period is due to the different dissection procedures used in each case (see Experimental and ref. [29]). The further increase in levels after the second day was, on the other hand, absolutely GA-dependent. In the starchy endosperm almost the whole of the increase in amino acid levels during germination was dependent upon the presence of the embryo or of GA (Fig. 1b). GA could not be replaced by compounds known to induce other aspects of metabolism in this experimental system [11], e.g. indole acetic acid, glutamine, hydroxylamine or combinations of indole acetic acid with glutamine or hydroxylamine (unrecorded data). The induction of increased free amino acid levels by GA was prevented by the inhibitors of protein synthesis cycloheximide and mikamycin (Table 2) suggesting that the response is dependent upon active protein synthesis.

Most of the individual amino acids, including the major ones glutamine and proline, responded to GA in the same way as the total free amino acid fraction. However, the levels of one amino acid, tryptophan, showed a distinctly different pattern of behaviour in the aleurone tissue (Fig. 2). In this case, removal of the embryo had little or no effect on the levels of the amino acid, but the addition of GA to incubating endosperms induced a sharp decline after the first day. Aspartic and glutamic acids showed similar changes to tryptophan, but their behaviour patterns were less well defined probably due to the difficulties associated with their accurate quantification.

The levels of the quaternary nitrogen compounds in aleurone tissue following removal of the embryo and the application of GA are shown in Table 3. The increase in the level of choline which occurred during germination was considerably depressed following removal of the embryo, and GA was partly able to replace the embryo. Thus, choline behaved like the total amino acids but less dramatically so. Glycine betaine had a behaviour pattern

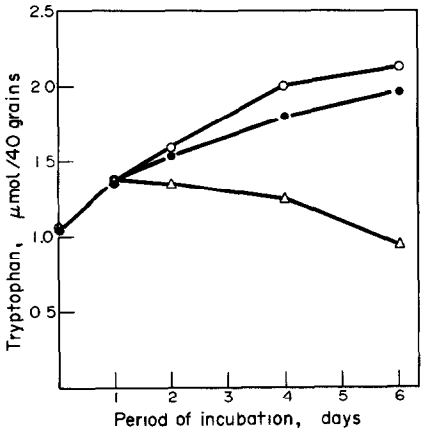

Fig. 2. Tryptophan levels in the aleurone tissue of wheat. ○, levels in germinated grain; ●, levels in incubated endosperms; △, levels in endosperms incubated with 1 μM GA.

Table 3. Effect of removing the embryo and of GA on the levels of quaternary nitrogen compounds in the aleurone tissue

	Choline μmol/40 grain	Glycine betaine μmol/40 grain
Ungerminated grain	0.43	8.96
Grain germinated 4 days	1.52	5.96
Endosperms (—embryo) incubated 4 days	0.81	10.6
Endosperms incubated 4 days in 1 μM GA	1.08	3.94

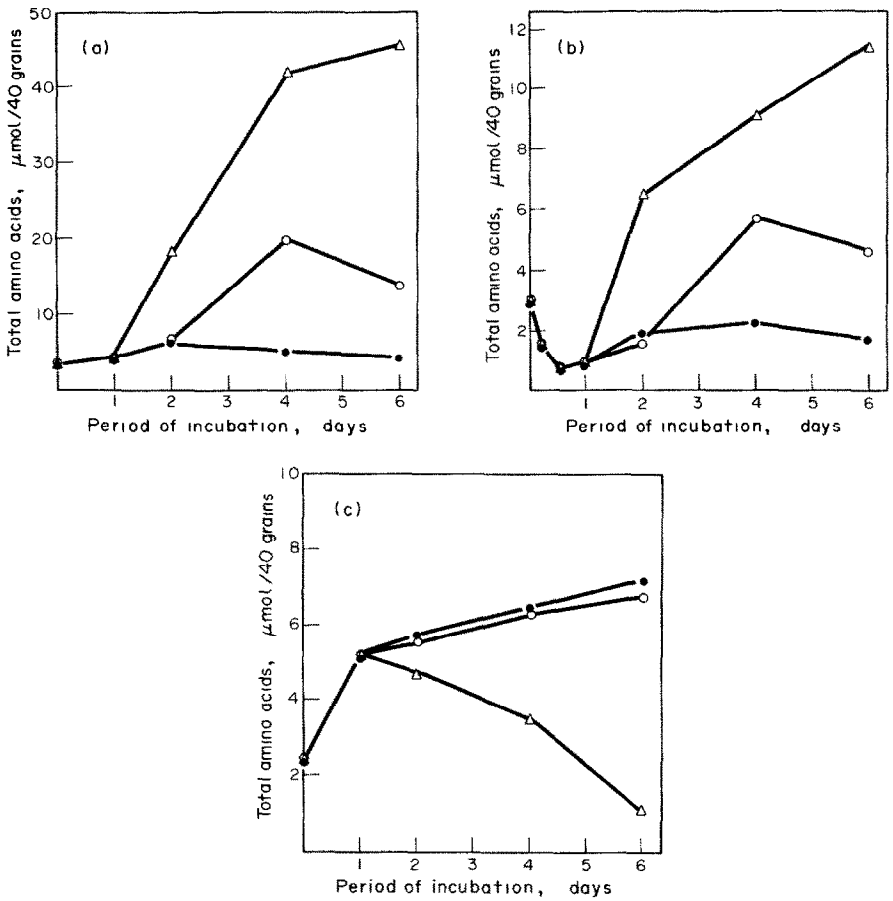

Fig. 3. Total amino acid levels in (a) the fast release fraction, (b) the slow release fraction and (c) the retained fraction from aleurone tissue. ○, tissue from germinated grain; ●, tissue from incubated endosperms; △, tissue from endosperms incubated with 1 μM GA.

which was almost exactly the inverse of that of the total amino acids, and more like that of tryptophan. Thus, removal of the embryo did not cause a fall in the level of glycine betaine (indeed, it caused a significant increase) and GA induced a large decrease.

Release of amino acids and quaternary nitrogen compounds from incubated aleurone tissue

Preliminary experiments not reported here showed that isolated aleurone layers, when incubated in a buffered aqueous medium, released amino acids and quaternary nitrogen compounds into the medium for at least 6 hr. Using a conductivity meter the release of electrolytes from isolated aleurone layers into the bathing medium could be monitored continuously. An initial rapid release was observed followed by a more gradual leakage of electrolytes from the tissue. It was found, by plotting the conductivity on a log scale and sequentially subtracting the pseudo-linear slowest phase from earlier release phases that a number of apparently separated phases could be recognized. This procedure is clearly based on efflux analysis of algal and higher plant tissues [11] but we would emphasize that we are not attempting to apply this rigorous method to the extremely complex aleurone tissue. This procedure revealed a number of very fast release phases, possibly two, with half-times of the order of 5–10 sec and a few min respectively, and a slower phase with a half-time exceeding 1 hr. As far as the amino acids and quaternary nitrogen compounds were concerned continuous recording was not possible, and the release plots for these compounds revealed only 2 phases with half-times of a few minutes (presumed to be the amalgamation of the first 2 phases seen in the conductivity experiment) and >1 hr. We have named the material in these phases the fast release fraction (measured over 0–15 min) and the slow release fraction (measured over 15 min–6 hr) respectively, and the results presented in this paper are discussed in terms of these 2 phases. In addition to the released compounds a third fraction could be identified and quantified by removing the aleurone layers from the incubation medium at the end of the fast release or at the end of the slow release period and homogenizing and extracting them. The amounts of total amino acids or quaternary nitrogen compounds contained in this third fraction were always equivalent at the end of the slow release period to those extracted at the end of the fast release period, and they presumably represent a constant pool of compounds held within the aleurone tissue. It will be referred to as the retained fraction. The slow release fraction must, therefore, represent principally the

Table 4. Effect of removing the embryo and of GA on the levels of amino acids and quaternary nitrogen compounds in the fractions from aleurone layers

	Ungerminated grain			Grain germinated 4 days			Endosperms incubated 4 days			Endosperms incubated 4 days with 1 μM GA			Wheat Gluten
	Fast release	Slow release	Retained	Fast release	Slow release	Retained	Fast release	Slow release	Retained	Fast release	Slow release	Retained	[13]
Amino acid results presented as the percentage of the sum of the amino acids determined:													
Aspartate	4.0	3.4	9.8	2.7	3.0	11.9	4.3	nd	9.3	0.6	1.5	nd	2.6 Aspartate + Asparagine
Asparagine + Threonine + Serine	20.7	24.6	9.3	12.9	25.6	10.4	19.3	nd	9.0	13.5	8.9	nd	2.5 Threonine; 4.7 Serine
Glutamate	4.3	1.2	3.5	2.5	1.2	17.5	14.1	nd	9.5	3.9	4.3	nd	34.0 Glutamate + Glutamine
Glutamine	17.7	24.6	22.7	30.7	24.5	12.6	18.1	nd	12.4	30.1	27.4	nd	
Proline	6.2	2.8	4.0	13.8	5.9	15.7	14.0	nd	20.0	7.8	4.0	nd	16.1 Proline
Glycine	2.9	5.0	3.5	1.3	3.8	1.0	2.1	nd	1.4	2.0	2.4	nd	5.5 Glycine
Alanine	8.8	8.5	4.4	7.2	8.6	4.3	12.0	nd	7.2	10.9	13.0	nd	3.5 Alanine
Isoleucine	1.4	0.9	0.4	4.7	3.8	nd	2.6	nd	0.8	3.5	3.6	nd	3.9 Isoleucine
Leucine	5.1	4.7	1.7	8.2	8.1	3.2	2.6	nd	0.6	8.0	10.3	nd	6.9 Leucine
Tyrosine	1.8	1.2	1.7	0.9	1.3	2.8	1.8	nd	2.6	2.6	3.6	nd	2.4 Tyrosine
Phenylalanine	2.5	1.8	0.8	6.1	4.9	0.7	1.5	nd	nd	5.3	5.3	nd	3.8 Phenylalanine
Histidine	3.3	2.8	1.7	2.5	4.1	3.0	1.8	nd	1.1	2.2	4.1	nd	1.8 Histidine
Tryptophan	13.3	11.7	33.4	2.4	nd	14.6	3.0	nd	24.5	3.9	2.3	nd	0.7 Tryptophan
Arginine	4.8	4.1	0.4	3.4	3.8	0.7	1.5	nd	0.3	4.6	8.1	nd	2.4 Arginine
Results presented as μmol/40 grain													
Aspartate	0.11	0.11	0.22	0.69	0.20	0.66	0.28	nd	0.57	0.30	0.17	nd	
Asparagine + Threonine + Serine	0.56	0.78	0.21	3.27	1.67	0.58	1.26	nd	0.55	6.11	0.97	nd	
Glutamate	0.11	0.04	0.08	0.61	0.08	0.97	0.92	nd	0.58	1.80	0.47	nd	
Glutamine	0.48	0.78	0.51	7.75	1.60	0.70	1.18	nd	0.76	13.6	2.97	nd	
Proline	0.17	0.09	0.09	3.48	0.39	0.87	0.91	0.22	1.22	3.56	0.44	0.22	
Glycine	0.08	0.16	0.08	0.35	0.25	0.06	0.14	nd	0.09	0.93	0.27	nd	
Alanine	0.24	0.27	0.10	1.83	0.56	0.24	0.78	nd	0.44	4.92	1.41	nd	
Isoleucine	0.04	0.03	0.01	1.20	0.25	nd	0.17	nd	0.05	1.61	0.39	nd	
Leucine	0.14	0.15	0.04	2.07	0.53	0.18	0.17	nd	0.04	3.63	1.12	nd	
Tyrosine	0.05	0.04	0.04	0.24	0.09	0.16	0.12	nd	0.17	1.18	0.39	nd	
Phenylalanine	0.07	0.06	0.02	1.54	0.32	0.04	0.10	nd	nd	2.43	0.58	nd	
Histidine	0.09	0.09	0.04	0.65	0.27	0.17	0.12	nd	0.07	1.00	0.45	nd	
Tryptophan	0.36	0.37	0.75	0.62	nd	0.81	0.20	nd	1.50	1.87	0.25	nd	
Arginine	0.13	0.13	0.01	0.88	0.25	0.04	0.10	nd	0.02	2.11	0.88	nd	
Total	2.70	3.16	2.24	25.24	6.51	5.53	6.50		6.10	45.1	10.8		
Choline	0.13	0.17	0.39	0.69	0.22	0.99	0.18	0.13	0.74	0.69	0.13	0.34	
Glycine betaine	1.17	1.82	7.12	1.59	2.34	3.70	2.34	2.40	7.66	2.99	1.11	0.95	

nd—not determined.
Asparagine, threonine and serine were not resolved in these analyses and are presented in a combined value.
Cysteine, valine, methionine and lysine were not determined in these analyses.

rate of production of amino acids and quaternary nitrogen compounds from another source or sources. This view was further supported by the observation that the sum of the fast release and retained fractions always equalled the total tissue content of amino acids. (Note that the experiments involving the incubation of isolated aleurone layers were carried out on a different seed sample to those in which the total tissue amino acids were determined and the data in Table 4 and Fig. 3 do not, therefore, compare closely with those in Table 1 and Fig. 1.)

The relative sizes of the fast and slow release fractions and the retained pools of the various nitrogen compounds changed markedly as germination proceeded. Since the parameters were also influenced by the removal of the embryo it was possible to study the factors which might control the pool sizes. The main features of the experimental results are presented in Fig. 3 and Table 4. The size of the fast release fraction of amino acids was low in the aleurone tissue of quiescent grain but increased after the first day of germination to reach a maximum at 4 days (Fig. 3a). Removal of the embryo prevented the increase and GA was able to replace the embryo in inducing the increase. Indeed, GA caused a dramatic increase in the fast release component to more than twice that found in the presence of the embryo. The pattern of changes in the slow release fraction of amino acids was similar to that of the fast release fraction except that there was a decline in the slow release fraction during the first 12 hours of germination (Fig. 3b). In contrast to the later increase in the size of this fraction the decline was independent of GA or any other factor emanating from the embryo. The size of the retained fraction of amino acids was low in the aleurone tissue of quiescent grain but doubled during the first day of germination (Fig. 3c). It continued to increase, but less rapidly, up to the sixth day. Removal of the embryo did not affect this pattern of development but the addition of GA to incubating endosperms induced a dramatic decline in the size of the fraction after the first day (cf. the pattern of tryptophan, Fig. 2). GA did not, however, influence the increase during the first day confirming that this increase occurred independently of control from the embryo. Table 4 contains the results for choline and

glycine betaine and the individual amino acids. Choline changes corresponded closely to those of the total amino acids (cf. Fig. 3) in that the fast release fraction increased during germination. This increase did not occur in incubated endosperms but GA induced an increase equivalent to that seen in the presence of the embryo. In the retained fraction choline increased two-fold during germination and also in incubated endosperms. Incubation of the endosperms in the presence of GA resulted in a much lower level. The levels of choline in the slow release fraction remained low and they did not show a clear behaviour pattern. The amount of glycine betaine in the fast release fraction did not change significantly during the germination of whole grain but increased slightly in incubated endosperms. The effect of GA was to slightly increase the value observed in the endosperms. Small changes also occurred in the slow release fraction where the level of glycine betaine increased during germination and in incubated endosperms, and GA induced a decrease. More significant changes occurred in the retained fraction where the level of glycine betaine decreased during germination. The decrease was prevented by removal of the embryo. GA substituted for the embryo and induced a large decrease in the level.

Analysis of the individual amino acids contained in the fast release, slow release and retained fractions revealed (Table 4) a somewhat more complex situation than that observed for the total amino acids. Data for the slow release fraction from the aleurone tissue of incubated endosperms and for the retained fraction from endosperms incubated with GA are not presented since insufficient amounts were obtained to allow accurate measurement in the automatic analyser.

For the sake of simplicity, only the major amino acids will be considered here. The first obvious feature of the data lay in the marked similarity between the composition of the fast release and the slow release fractions and the contrast between them and the retained fraction. In the ungerminated grain asparagine + threonine + serine, glutamine and tryptophan were the major amino acids in the fast release and the slow release fractions. At the fourth day of germination the overall composition of the slow release fraction showed few changes; the proportion of proline was, however, doubled and that of tryptophan was very much lower so that it was no longer a major amino acid. In the fast release fraction the proportion of tryptophan had declined similarly and there was also a significant decline in the proportion of asparagine + threonine + serine and an increase in the proportions of glutamine and proline. Proline was now a major amino acid in this fraction. The decline in the proportion of asparagine + threonine + serine and the increase in the proportion of glutamine observed during germination did not occur in endosperms incubated for the same period of time but incubation of the endosperms with GA induced changes in their proportions similar to those observed in the germinated grain. On the other hand, the changes in the proportions of tryptophan and proline during germination were not affected by removal of the embryo or by GA. In complete contrast, the proportion of glutamic acid in the fast release fraction was similar in the quiescent and in the germinated grain but in the incubated endosperm its proportion increased so that it was a major contributor to the fraction. Incubation of the endosperms with GA maintained the proportion of glutamic acid at the level found in germinating grain. At the same time GA action considerably reduced the contribution of asparagine + threonine + serine although their combined contribution did not change significantly during germination.

In all the samples analysed the retained fraction was characterized by much higher proportions of aspartic acid and tryptophan than the fast release and slow release fractions. Within this general feature the retained pool also underwent some changes during germination. For example, the proportions of glutamic acid and proline increased and those of glutamine and tryptophan decreased. Similar changes in glutamic acid, glutamine and proline were also evident in incubated endosperms suggesting that they occurred independently of any hormonal factor from the embryo. On the other hand, the proportion of tryptophan remained high compared with the lowered proportion in germinated grain; the contribution of this amino acid is, therefore, clearly under some sort of control from the embryo.

To summarize, it is clear that the various amino acids in the fast release, slow release and retained fractions are affected differently by the embryo and by GA. In particular, the analysis of the whole tissue reported above identified tryptophan, aspartic acid, glutamic acid and glycine betaine as having patterns of change distinct from those of the other amino acids and choline. Tryptophan and glycine betaine in particular, and aspartic acid and glutamic acid to a lesser extent, behaved like the total amino acids of the retained fraction. The data in Tables 1 and 4 can explain the differences. Thus, the retained fraction contains most of the tissue tryptophan, aspartic acid, glutamic acid and glycine betaine. The character of this fraction would, therefore, be expected to dominate the behaviour of these compounds in the whole tissue analyses. On the other hand, the other amino acids and choline are distributed more to the fast release fraction and since this fraction is considerably larger than the retained fraction, its character can be expected to dominate the behaviour of these other amino acids and of choline in the whole tissue analyses.

DISCUSSION

The embryonic and aleurone tissues of the germinating wheat grain have high synthetic activities supported by the mobilization of stored nutrients. In this situation, the free amino acids represent an amphibolic pool or pools of material with rapid turnover rates. Thus, they are produced by hydrolysis of reserve protein, consumed by incorporation into new protein and, in the case of the aleurone tissue, lost by efflux from the tissue. In addition many of the amino acids can be expected to exchange rapidly with the general intermediary metabolism. The starchy endosperm, being a dead tissue, will have a single pool of amino acids (although not necessarily equally distributed through the tissue) produced by the hydrolysis of its own reserve protein and by release from the aleurone tissue and lost by uptake into the embryo. The tissue pools of glycine betaine and choline in the grain will similarly represent the balance between production and consumption. Without data on these kinetic parameters, a comprehensive account of the complex events associated with this aspect of nitrogen metabolism cannot be presented. In the case of the aleurone tissue, however, it is known that GA

stimulates the incorporation of (radio-labelled) amino acids into protein [13] and the experiments reported here have shown that the hormone increases the loss of amino acids from the tissue. The increased tissue content of the free amino acids resulting from GA action must, therefore, be achieved by increased production from stored protein. This is further supported by the fact that the composition of the free amino acids in the fast and slow release fractions reflects that of the glutens (Table 4). The high proportions of glutamate + glutamine and proline are particularly characteristic of the glutens [14] and of these fractions. The aleurone tissue is reported to contain about 20% of its total protein as glutens and the total protein of the tissue contains a high proportion of glutamine which is characteristic of the glutens [14].

The presence of high levels of glutamine in the endosperm tissues during germination and in incubating endosperms does not support the suggestion made in our earlier publications [11] that glutamine might play an important role in activating enzymes such as phytase. It is more likely that the effects of glutamine seen in those experiments were the result of some non-specific effect of the added glutamine.

The GA-induced increase in choline levels in aleurone tissue is surprising since the incorporation of (radio-labelled) choline into phosphatidyl choline is not affected by GA [15, 16] and the most active period of incorporation occurs early during germination before the action of GA [15]. Perhaps the increase in choline is an indirect result of GA action on the levels of choline precursors such as glycine and serine, but more definitive experiments will be necessary to clarify this tissue. The presence of large quantities of the other quaternary nitrogen base, glycine betaine, is of more obvious relevance. Glycine betaine is characteristic of water-stressed plant tissues in which high concentrations of salt occur [17]. Much recent evidence suggests that the salts are concentrated in the vacuole [18, 19] while the glycine betaine is largely confined to the cytoplasm [20]. It has been proposed [20] that glycine betaine acts as a non-toxic cytoplasmic osmoticum maintaining an equal osmotic potential across the tonoplast and allowing the potentially toxic salts to be largely excluded in the vacuole. The partially dehydrated but viable aleurone and embryo tissues of the wheat grain during quiescence and early germination may be considered as specially adapted to water stress and in this context it is interesting to note the high concentrations of glycine betaine found in these tissues in contrast to that of the starchy endosperm. If this analogy is pursued the vacuole may be represented by the aleurone grains [21] which are known to be the repositories of the wheat grain's mineral and protein reserves. Since glycine betaine was found mainly in the retained fraction in the aleurone tissue a tentative interpretation can be made regarding the locations of the fast release, slow release and retained fractions. In our preliminary experiments using the conductivity meter, some 70–80% of the electrolytes in the fast release fraction were washed out in the first minute. Similarly the loss of the amino acids and glycine betaine in this fraction occurred largely within the first minute. The total electrolyte release during this time was temperature independent suggesting a simple washing process. Furthermore, there was a close correlation between the quantitative and qualitative changes in the fast release fraction and those of the starchy endosperm (Figs. 1b and 3a). On the other hand, the fast release fraction of amino acids was disproportionally large in comparison with the amounts found in the whole of the starchy endosperm. This could, however, be accounted for by the concentration of the storage protein in the regions adjacent to the aleurone layer. Then it may be surmized that the fast release fraction is composed mainly of cell wall free space and surface material in close contact with the starchy endosperm. The slow release fraction would then represent the release or leakage of material through the plasmalemma while the retained fraction includes the cytoplasmic and probably other cellular pools. It may be noted in passing that this latter fraction, in contrast to gluten and to the fast and slow release fractions, is high in tryptophan.

If this tentative interpretation is correct then a number of interesting suggestions may be made regarding the role of the plasmalemma in controlling the release of compounds from the aleurone tissue and the possible influence of GA on it. The different compositions of the slow release fraction and the retained fraction and the similarity of the former to that of reserve protein may be interpreted in at least two ways. Either the plasmalemma exercises selective control over efflux so that tryptophan, aspartic acid, glutamic acid and glycine betaine are preferentially retained while glutamine is released or the hydrolysis products from the aleurone grain are exported through the plasmalemma possibly by some lysosomal route without equilibration with the retained cytoplasmic pool. The latter possibility is particularly attractive in view of other evidence [11] that GA action in the aleurone tissue is concerned with lysosome formation. Since GA treatment collapses the retained fraction, it appears that GA, either directly or indirectly, increases the permeability of the plasmalemma. Since the effect is dependent upon protein synthesis it would appear to be a secondary action of the hormone.

The decline in the size of the slow release fraction of amino acids during the first few hours of germination is also interesting in the context of this discussion since it implies a decreased permeability of the plasmalemma. Essentially similar results to the present ones have been previously reported regarding the release of mineral ions and reducing sugars from the aleurone tissue of wheat [22, 23] and a similar situation regarding the induction by GA of increased release of amino acids, mineral ions and sugars from barley aleurone tissue has been described [24–26].

In the efflux experiments glutamine and glycine betaine accounted for about 50% of the nitrogen released from the aleurone tissue in the form of amino acids and quaternary nitrogen compounds. This suggests that the compounds are important vehicles for nitrogen transport from that tissue during germination. It has been suggested before [27] that the aleurone tissue is a significant source of amino acids for the seedling during germination. Whatever the proportion of amino acids arising from the aleurone tissue and the starchy endosperm during germination glutamine is a major contributor in each case. On passing to the seedling, therefore, its nitrogen component must be transposed to asparagine which is the principal amino acid of that tissue (cf. the conversion of glucose to sucrose during the transport of sugars from the endosperm to the seedling [28]).

EXPERIMENTAL

Plant material. Grains of the soft winter cultivar Capelle Desprez were used in batches of 2.3 g (40 grains). The sterilization, incubation and dissection of the plant material has been described previously [29].

Extraction of amino acids. Plant material was boiled for 1 min in 20 ml EtOH to destroy enzyme activity, homogenized for 1 min using a top-drive homogeniser and filtered through sintered glass. The residue was further homogenized and extracted × 2 in 20 ml EtOH–H_2O (3:1). The bulked filtrates were reduced to a small vol at 40° in a rotary evaporator and taken up in 50 ml EtOH–H_2O (4 1). This was centrifuged to remove precipitated gliadin and applied to a 2 × 0.8 cm column of Zeo carb 225 (H^+ form in EtOH–H_2O (4.1)) [30]. The amino acids were eluted from the column with 0.1 M NaOH, neutralized with conc HCl and stored in the dark at −25°.

Quaternary N_2 compounds were extracted from separate samples of plant material according to the method described in ref. [17].

Release of compounds from isolated aleurone layers. Aleurone layers were incubated in 10 ml 0.1 M Na Pi buffer, pH 7.0 in a 100 ml flask. The flask and contents were incubated at 25° in a reciprocating H_2O bath. After 15 min the incubation medium was decanted off for analysis of the fast release fraction (see Results). The aleurone layers were quickly washed × 3 with dist H_2O, damp dried on filter paper, placed in a fresh 10 ml incubation medium and incubated further. Aliquots of the medium were taken at intervals up to 6 hr for analysis of the slow release fraction. At the end of either the 15 min or the 6 hr incubation (different samples) the aleurone layers were extracted for analysis of the retained fraction.

Determination of amino acids and quaternary nitrogen compounds. Total amino acids were determined using the ninhydrin method [31]. Individual amino acids were determined using an amino acid analyser linked to a computer data processing system [32]. The analyser did not resolve asparagine and glutamine from serine and threonine and, therefore, samples were analysed before and after hydrolysis for 2 hr at 100° in 4M HCl. Asparagine and glutamine could then be determined by difference on the aspartate and glutamate peaks, and serine and threonine suffered no interference in the chromatography of the hydrolysed samples. Proline was determined separately in unhydrolysed samples by the method of Singh *et al.* [33]. Choline and glycine betaine were determined using method described previously [17]. Each experiment was conducted in duplicate and each analysis was done in duplicate at least. Thus, each value recorded in the results is the average from several determinations.

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