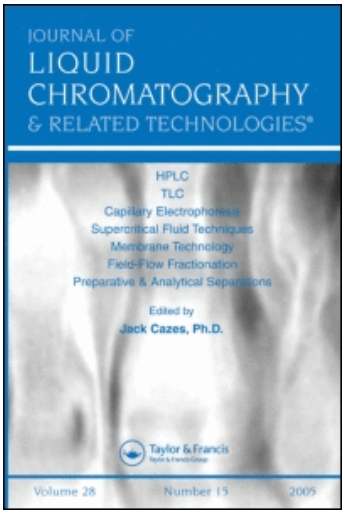

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PEPTIDE BIOSYNTHESIS AND CHANGES IN FREE AMINO ACID COMPOSITION IN DEVELOPING BEAN SEEDLINGS *(Phaseolus vulgaris)*

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ABSTRACT

Bean seedling homogenates incorporated labelled [^{14}C] aspartate, [^{14}C] glutamate and [^{14}C] glycine into a number of hydrolyzable radiopositive compounds, as detected by an X-film technique from the thin-layer chromatography plates. The original labelled amino acids in these compounds could be identified by re-chromatography of their acid hydrolysates. The structures of these compounds are still under study, but they seem to be short-chain tri- pentapeptides, synthesized apparently directly from amino acids by the cytoplasmic peptide synthetases. Both dry and swollen beans contained considerable concentrations of free amino acids, the amounts of which mostly decreased as the seedlings grew, although the concentrations of glutamine and γ-aminobutyric acid increased.

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INTRODUCTION

The germination of seeds involves a number of physiological and biochemical processes, the purpose of which is to guarantee a supply of nutrients and other growth factors to the developing seedlings (1-2). Mobilization of the storage carbohydrate and protein polymers (3-5), changes in enzyme activities (6-8), changes in the concentrations of free amino acids (9,10), increased activities of amino acid and peptide transport (11-13,24) and the appearance of a number of peptides (9,14) have been well characterized. A number of biosynthetic processes also occur (2), but these are less well known. This is particularly the case with the peptides, although a great number of peptides has been observed to occur in seeds and seedlings (14-16).

A large proportion of the plant peptides are probably products of γ-glutamyl transpeptidase (γ-GTP, EC 2.3.2.2) in a cellular membrane fraction (17-19), while another possibility is formation by cytoplasmic peptide synthetases, which can form simple peptides rapidly and directly from amino acids. Observations on the possibility of this direct peptide synthesis in bean seedling homogenates are made here using a very sensitive X-film technique (20). The cellular environment for natural peptide biosynthesis is monitored by analyzing the composition of the free amino acid pools.

EXPERIMENTAL

Commercial beans (Phaseolus vulgaris), which had been stored in dry bags at room temperature, were germinated between wet filter papers and the seedlings grown in a medium containing (g/l) 0.8 KNO_3, 0.8 $Ca(NO_3)_2$, 0.2 $MgSO_4$ and 0.2 KH_2PO_4. For the study of peptide biosynthesis the beans and seedlings were homogenized in 25 mmol/l potassium phosphate buffer (pH 7.4) containing (mmol/l) 50 KCl, 5 sodium phosphate, 10 $MgCl_2$, 10 glucose, 0.1 cAMP, 0.5 ATP, 0.26 phosphoenolpyruvate and 10 mg/l pyruvate kinase. The incubation mixtures also contained 0.25 mol/l of each of the 20 protein amino acids and 185 MBq/l of [^{14}C]glutamate (specific activity 10.8 TBq/mol), [^{14}C]aspartate (9.0 TBq/mol) and [^{14}C]glycine (9.3 TBq/mol). All the radiochemicals were purchased from Amersham International Limited, Amersham, U.K.

Incubations were performed at 310 K for varying periods, but 1 h was observed to be the optimal time for peptide synthesis. The reactions were stopped by adding 0.1 mol/l cold perchloric acid. The resulting protein precipitates were removed by centrifugation. The extracts were then neutralized with KOH, the potassium perchlorate centrifuged out and the residues lyophilized to dryness. The residues were dissolved in distilled water and applied to the TLC plates. The chroma-

tograms were developed with 70 % (v/v) ethanol and 75 % (w/v) phenol in water. The dry plates were then tightly covered with X-films and left for one week in the dark at room temperature. They were then developed with Kodak DX-80 and fixed with Kodak FX-40. Some of the samples were hydrolyzed with 6 mol/l HCl at 393 K for 20 h, the hydrolysates being evaporated to dryness and analyzed as above. Some of the TLC plates were sprayed with ninhydrin (15 % ninhydrin in 3 mol/l potassium citrate buffer, pH 5.1), and some tests were carried out on the effect of chloramphenicol on the peptide biosynthesis as above, but with the addition of 2.05 mmol/l chloramphenicol to the incubation mixture.

Free amino acids from the beans and bean seedlings were extracted with 5 % (w/v) trichloroacetic acid (TCA), which was then removed by shaking with diethyl ether, and the residues were lyophilized and dissolved for examination in an automatic amino acid analyzer (Kontron Liquimat III).

RESULTS AND DISCUSSION

When three labelled amino acids, [^{14}C]glutamate, [^{14}C]aspartate and [^{14}C]glycine, those which are generally abundant in peptides (21) were used in combination as markers in the amino acid mixture, the label appeared in a number of new radiopositive spots after

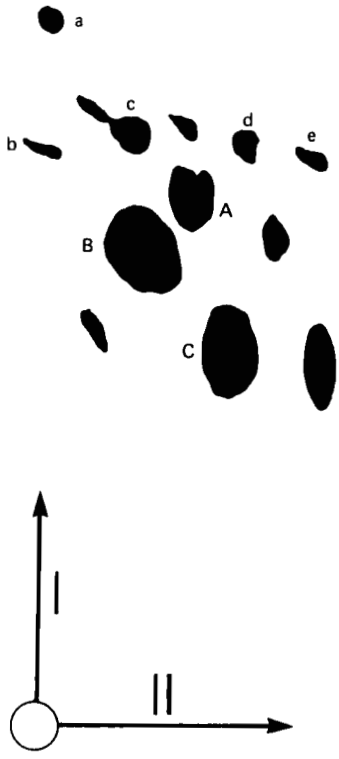

Figure 1. Autoradiographic X-film of the labelled spots formed from the original labelled amino acids (A = [^{14}C]-glutamic acid, B = [^{14}C]aspartic acid, C = [^{14}C]glycine) during 1 h incubation of bean seedling homogenates at 310 K. The hydrolyzable spots (a-e) were scraped up and studied by re-chromatography after hydrolysis in 6 mol/l HCl at 393 K for 20 h.

incubation of the bean seedling homogenates. Some of these spots disappeared after hydrolysis with 6 mol/l HCl. Although the original labelled amino acids gave rise to a number of simple metabolic derivatives (γ-aminobutyric acid, glutamine, asparagine, serine, tricarboxylic acid cycle intermediates), the possible peptides formed during incubation were sought among the unidentified hydrolyzable spots. Five such spots (labelled a-e in Figure 1) were discovered. Re-chromatography after hydrolysis showed the presence of the original labelled amino acids and [^{14}C]serine (probably formed

TABLE I

Numbers of radiopositive spots formed from [^{14}C]glutamate, [^{14}C]aspartate and [^{14}C]glycine in bean seedling homogenates during 1 h incubation at 310 K

Products	Labelled amino acids added: [^{14}C]Glutamate (A), [^{14}C]Aspartate (B), [^{14}C]Glycine (C)		Original labelled amino acids identified in the spots
Hydrolyzable	5	a	-
		b	aspartate, glycine
		c	glutamate
		d	glycine (+ serine)
		e	glutamate
Non-hydrolyzable	4		
Formed in hydrolysis	2		

For the location of the original labelled amino acids (A-C) and the hydrolyzable spots (a-e), see Figure 1. [^{14}C]Serine was apparently formed from [^{14}C]glycine during incubation.

from [^{14}C]glycine) in these compounds (Table 1). The exact structures of these compounds are still unknown, but studies on structurally better defined peptides (22) and on the R_f values for the ninhydrin-positive compounds of the bean seedling extracts, suggest that they are simple tri-, tetra- and pentapeptides. Chloramphenicol had no effect on the peptide biosynthesis in

TABLE II

Concentrations of certain free amino acids in beans and bean seedlings during germination

Amino acid	Concentration (μmol/kg wet weight) per original bean: dry bean	swollen bean (2 d)	per seedling: seedling (12 d)
Increased:			
γ-Aminobutyric acid	485 ± 136	512 ± 91	523 ± 25
Glutamine	175 ± 21	356 ± 101	513 ± 19
Decreased:			
Arginine	2607 ± 430	1156 ± 306	260 ± 58
Aspartic acid	909 ± 182	393 ± 155	253 ± 19
Glutamic acid	866 ± 199	487 ± 89	271 ± 24
Proline	621 ± 174	241 ± 83	46 ± 13
Alanine	366 ± 158	218 ± 54	234 ± 40
Valine	471 ± 149	328 ± 109	35 ± 8
Histidine	252 ± 28	195 ± 33	21 ± 2
Lysine	50 ± 19	45 ± 21	4 ± 0.4
No changes:			
Asparagine	1170 ± 284	1136 ± 301	1028 ± 89
Glycine	223 ± 80	78 ± 9	214 ± 28
Serine	99 ± 30	121 ± 36	72 ± 3

Results are means (± S.E.M.) from 4-6 experiments.

the bean seedling homogenates, indicating a cytoplasmic place of the synthesis. As concluded earlier (22), this type of the peptide biosynthesis may take place without any coding by DNA. The biosynthetic ability in beans started during their swelling and lasted for the whole development time of the seedlings.

The beans (both dry and swollen) and bean seedlings contained relatively high concentrations of free amino acids and other ninhydrin-positive compounds (Table 2), the main constituents being arginine, asparagine, aspartic and glutamic acids, glutamine and γ-aminobutyric acid, giving a good intracellular environment for peptide biosynthesis. The concentrations of free amino acids mostly decreased markedly as germination progressed, but the amounts of glutamine and γ-aminobutyric acid increased slightly, while the amount of asparagine, serine and glycine were kept at relatively constant level. These analysis results are in general equal, both qualitatively and quantitatively with those reported earlier in germinating leguminous and other seeds (1,5, 7-9,23).

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