A SURVEY OF AMINO ACID CONTENT OF THE UREDOSPORES OF SOME RACES OF WHEAT RUST (PUCCINIA GRAMINIS)[1]

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ABSTRACT

The free and combined amino acids of seven samples of wheat rust were analyzed quantitatively by paper and column chromatography and compared with the amino acids present in uninfected wheat leaves. All races studied contained different proportions of free amino acids.

INTRODUCTION

The physiology of *Puccinia graminis* has been studied in some detail. For example, investigations of wheat leaf metabolism have indicated that diffusible substances spread from the fungal mycelium (1) and, as the parasite developed, the rise in oxygen consumption in the tissue of non-resistant wheat was found to occur later than in rust-resistant plants (2). This increase in respiration proved to be not merely an additive effect of the fungus respiration, but to be due to respiratory changes in the host tissues (3). In rust-susceptible wheat, decreased glycolic acid oxidase activity was shown to accompany the increased respiratory rate (4).

In contrast, the chemical constitution of the uredospores of *P. graminis* has been little investigated. The carotenoid pigments of the uredospores have been described (5) and studies made of a substance (6) or substances (7) that cause differentiation of infection structures. An examination of four races of wheat rust has shown a different total amino acid and sugar content for each race (8).

In the present work, the free and combined amino acids of seven samples of wheat stem rust uredospores are analyzed quantitatively by paper and column chromatography and compared with the free amino acids present in uninfected wheat leaves.

MATERIALS AND METHODS

The original rust cultures were obtained from the Plant Pathology laboratory in Winnipeg. The two-race mixtures and one sample of race 29, as well as the samples of field-collected wheat rust spores, were grown in Winnipeg. All races except the field-collected mixtures were grown on Little Club wheat in a greenhouse. Spores of all races were harvested when they reached maturity.

The free amino acids were obtained from the spores in the following manner: The uredospores were first extracted with ethyl ether for 20 hours to facilitate penetration of the aqueous solvent. Boiling water (20 ml per g) was then added to the sample and refluxed for 1 hour. The mixture was filtered, the filtrate cooled and poured into two volumes of ethanol. Any precipitate was removed by filtration. The extract was taken to small volume under reduced pressure, and the concentrate placed on a short (20 g) column of Dowex 50, 4% cross-linked, (50X4), 50–100 mesh, prepared according to Partridge (9, 10), and washed with 200 ml water. Acidic and neutral fractions were thus washed through, and the amino acid fraction (plus other weakly basic compounds) was then eluted from the Dowex 50X4 column with 0.2 *N* ammonium hydroxide. The ammonia was removed under reduced pressure and then *in vacuo* at 50° C.

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A sample of uninfected leaves of Little Club wheat was freeze-dried and extracted in the same manner as the spores.

Individual amino acids were identified qualitatively by two-dimensional chromatography on Whatman No. 1 chromatographic paper. The sample was developed first with 80% phenol containing 0.04% 8-hydroxyquinoline, a small beaker of dilute ammonia being placed in the cylinder (11). The second solvent system was *n*-butanol – acetic acid – water 4:1:1 (12). The chromatogram was sprayed with 0.25% ninhydrin in *n*-butanol (13) to develop the color, or with isatin (14) to show proline and hydroxyproline and to help identify other amino acids by color differences between the effects of the two sprays.

The amino acid composition of the various races was determined as described by Moore and Stein (15). The concentration of amino acid in each fraction was estimated colorimetrically according to Moore and Stein (16) and Chinard (17).

Bound amino acids were released from the residue of the aqueous extraction by a 20-hour hydrolysis in 6 *N* hydrochloric acid used in the proportion of 25 ml per g of original sample (18, 19). The hydrolyzate was filtered and the hydrochloric acid removed from the filtrate by evaporation under reduced pressure. After the removal of most of the hydrochloric acid, a small amount of fine precipitate was present in the aqueous solution. This substance was slightly soluble in the Moore and Stein buffer pH 3.42 used initially on the column and, unless it was removed, the separation of the amino acids was not distinct. The hydrolyzate, after filtration (or centrifugation), was taken to dryness and stored over sodium hydroxide pellets to remove any remaining traces of free acid. An aliquot of suitable size (representing 0.2-g uredospores) was dissolved in buffer pH 3.42 and placed on the Moore and Stein column as in the case of the extract.

The individual amino acids in both the extract and hydrolyzate were identified by paper chromatography and by their position in the series of fractions from the Moore and Stein column. To calculate the percentage composition of a mixture of amino acids, the weight of each acid was expressed as a percentage of the total weight of the amino acids recovered from the column. This procedure was adopted to give a uniform basis of comparison among races.

After the over-all pattern of amino acid distribution had been noted, a synthetic mixture of amino acids was analyzed chromatographically to determine any limitations in the analytical methods.

RESULTS AND DISCUSSION

Each amino acid component of the synthetic mixture is recovered in essentially the same percentage of the total weight of amino acids in which it was present in the original mixture. When 10 mg of amino acids were applied to the column, 10.49 mg were recovered for an over-all efficiency of 104.9%.

By summing the weights of the amino acids in the fractions collected, the following information on over-all amino acid composition of *P. graminis* uredospores was obtained. Free amino acids made up approximately 0.5% of the original weight of spores and acid hydrolysis released a further 11% by weight of combined amino acids.

The proportions of free amino acids in the various races of rust studied are shown in Table I. Using the precision of recovery of the synthetic mixture as a model, appreciable differences in free amino acids were found between races. The variations in glycine and α-alanine contents separately, however, should be viewed with caution as the large amount of α-alanine present caused an overlapping of the peaks. There is also lack of resolution on the column of the peaks of tyrosine and phenylalanine.

TABLE I
Free amino acids

Amino acid	Race 11	11+15B	15B	15B+87	29	48	56	A*	B*	Little Club leaves
Methionine sulphoxide	—	—	—	—	—	—	—	4.4	—	—
Cysteic acid	8.2	9.9	9.3	5.8	9.7	8.4	12.4	6.3	10.2	—
Aspartic acid	5.2	5.3	5.0	7.1	8.4	5.0	12.6	17.8	4.9	3.8
Threonine	—	—	—	2.7	—	2.5	—	—	—	3.2
Serine	13.0	7.0	6.6	4.5	7.0	10.5	1.5	6.4	7.8	9.1
Glutamic acid	10.6	3.9	1.2	7.1	3.0	17.0	—	1.9	5.7	5.6
Proline	Trace	—	—	—	—	—	Trace	—	—	5.6
Glycine	5.2	1.8	10.4	0.2	0.3	3.3	0.6	0.6	1.3	0.8
α-Alanine	22.0	37.6	28.0	31.2	29.7	18.6	41.3	19.4	18.2	17.6
Valine	4.1	2.3	3.0	4.0	4.3	4.0	2.8	3.6	4.0	4.9
Methionine	2.5	1.8	—	1.0	1.3	1.7	0.6	—	0.5	2.0
Isoleucine	1.7	1.3	1.3	1.5	1.6	1.7	0.7	1.2	1.2	2.3
Leucine	2.1	1.5	1.4	1.6	2.1	2.0	3.0	1.6	1.4	3.3
Tyrosine	2.6	1.5	1.8	4.0	2.6	3.5	1.7	2.2	3.4	3.8
Phenylalanine	2.6	1.7	2.7	2.8	4.0	2.3	3.8	3.0	2.0	6.2
γ-Aminobutyric acid	13.1	18.6	22.6	22.0	18.0	11.4	12.7	29.1	29.3	21.1
Histidine	2.7	1.8	Trace	2.4	4.4	2.7	2.8	0.9	3.1	3.9
Lysine	3.0	2.0	2.8	0.7	1.8	2.1	2.3	1.5	1.3	4.7
Arginine	1.4	2.0	4.1	1.6	1.8	3.4	1.4	Trace	5.3	2.2

*A Stem rust, field-collected and stored before analysis.
*B Stem rust, field-collected.

The greatest variation is shown by glutamic acid and aspartic acid. α-Alanine and glycine together show considerable variation although less is shown by the other α-amino-monocarboxylic acids, i.e. valine and the leucines. The other main constituent, γ-amino-butyric acid varies considerably as does serine while somewhat less variation is shown by the basic amino acids and those containing a benzene nucleus.

In contrast to the free amino acids, the bound amino acids in *P. graminis* uredospores are remarkably constant in composition from race to race.

On the basis of the results presented here, it would appear that the free amino acid content of uredospores, although its quantitative determination is tedious, might be considered a useful method for differentiating among races of wheat rust. For example, race 56 might be identified through its lack of glutamic acid and high content of aspartic acid and α-alanine. Similarly, the presence of large amounts of glutamic acid and low content of α-alanine and γ-aminobutyric acid might be used to identify race 48.

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