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Abstract

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There are many reports concerning the determination of microbial amino acid pools. They differ in techniques used for extraction and in methods of amino acid analysis. In our laboratory we hove studied some extraction techniques for routine estimation of free amino acids in mycelio of Neurospora grown in a liquid medium. Mycelia of about 1 g. dry weight were washed repeatedly with cold distilled water and then ground in a mortar, with sand and the following extracting agents, to release all soluble amino acids and to precipitate the proteins: (1) 0. IN HCl/acetone (1:4, v/v, 0°C). (2) ethanol (96%)/water (5:1, v/v, 0°C) after homogenization the pH was adjusted to 7.0 and the homogenate was steamed for 20 min. (3) Perchloric acid (0.7N, 20°C). (4) Trichloroacetic acid (10%, w/v), followed by extraction of trichloroacetic acid with ether. (5) Distilled water (100°C for IO min.), using fresh mycelia as well as mycelia dried at 70°C for 16 hours.

After extraction, the homogenates were centrifuged and washed twice with the corresponding extracting agent by centrifugation. The supernatant and washings were removed by decanting, combined and evaporated under partial vacuum to dryness and taken up in 4-5 ml of distilled water. From this volume lipids were extracted twice with 10 ml of chloroform. After evaporation of the remaining water to dryness, the residue was taken up in 5.0 ml of 0. IN HCl. Aliquots of the solution were brought to pH 2.0 or 7.0 and diluted to a final volume dependent upon the dry weight of the mycelia from which it was extracted (~ 150mg/ml).

Water extraction of dried mycelia, as used by Neidleman and Kemmerer (1960 Arch. Biochem. Biophys. 88: 172), did not give exact results because there was on increase of free amino acids, probably by partial proteolysis during drying. Qualitatively and quantitatively similar results were obtained by all other extraction procedures investigated, including most commonly-used techniques. The most uniform results we obtained by extraction with ethanol-water, as used by Fuerst and Wagner (1957 Arch. Biochem. Biophys. 70: 311).

Investigations of free amino acids in Neurospora have been mode by one- or two-dimensional paper chromatography and by microbiological assays. In our laboratory we used a commercial automatic recording apparatus for the determination of amino acids by ion exchange chromatography (Bender and Hobein, Munich), described by Hannig (1959 Clin. Chim. Acta 4: 51). The columns and buffers used don't allow clear separation of glutamine and asparagine from serine. Therefore we hydrolyzed the amides at pH 2.0 by steaming for 30 min. After hydrolysis, ammonia and glutamic + aspartic acid are increased. Concentrations of glutamic and aspartic acid measured are the sums of free acids and their amides (individual amounts can be calculated from differences analyzed before and after hydrolysis). Major constituents of the pool are alanine and glutamic acid (+ glutamine); other amino acids show very low concentrations. Therefore it was necessary to carry out two assays of each extract, equivalent to 30-50 and 120-150 mgm/ml, respectively. The volumes of sample placed on the columns were 0.2-1.0 ml of the extracts.

With all stocks of Neurospora which we have analyzed, the record showed the presence of more than 30 reproducible components, large and small, including all proteinogenic amino acids (without tryptophan). There were distinct peaks corresponding to ornithine, ammonia, cystathionine, glucosamine, ß-alanine and some unknown maxima. To identify the peaks and to test their purity the eluates of columns were fractionated. The fractions of each peak were combined and, after desalting by ion exchange (Dowex 50-X4), evaporated to dryness. The residues were token up in a small volume of water for application to paper chromatography as described by Matthias (1961 J. Chromatog. 6: 333). Aliquots of the samples were applied to wedge-strip papers (4 cm x 50 cm, Schleicher and Schuell 2043 b mgl), which were developed by the ascending technique with n-butanol-acetic acid-water (4:1:1). After drying, chromatograms were stained with ninhydrin, Components present were identified by comparison with the position of authentic amino acid markers. All the peaks of proteinogenic amino acids found by ion exchange chromatography proved to be unitary.

To determine the amount of peptides present in extracts, HCl was added to a final concentration of 6N. The peptides were hydrolyzed for 8 hours at 120°C. After hydrolysis proteinogenic amino acids increased to different extents. Non-proteinogenic amino acids didn't change, nor did some of the unidentified peak. Three of the unknown maxima disappeared. In growth phases mycelia contained low quantities of peptides; in autolyzing mycelio they increased. • • • Physiologisch-chemisches Institute der Universität, Leipzig, Germany.