Elemental Analysis of Vitamin-Free Casamino Acids

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The elemental composition of three lots of vitamin-free Casamino Acids (Difco) was determined using X-ray fluorescence, atomic absorption, and colorimetric techniques.

Complex nitrogen sources are frequently used in the growth of fastidious microorganisms and insect cell lines. Although these sources are added to media primarily because of their organic nitrogen and growth factor components, their inorganic components, which are generally undetermined, may play vital roles. Grant and Pramer (2) demonstrated that the ash of yeast extract was required for the growth of the fungus, Arthrobotrys conoides Dreschler, and found that zinc was the essential component. Grimm and Allen (3) found that the addition of yeast extract to growth media promoted the production of cytochromes in Ustilago sphaerogena Burrill and that the level of zinc in yeast extract could account for the stimulation.

During an investigation of the nutritional requirements of the aquatic phycomycete, Catenaria anguillulae Sorokin (4-6), vitamin-free Casamino Acids (Difco) was used as the initial nitrogen source. In an experiment on the effects of the deletion of individual trace elements from a medium containing Casamino Acids, it was found (6) that growth was prevented by the omission of calcium or magnesium from a standard micronutrient supplement and limited by the omission of zinc or iron. The optimum concentration of phosphate for growth was determined by varying the phosphate level in a basal medium (6) using two nitrogen sources, Casamino Acids, and a defined amino acid mixture, in parallel series. By comparing the dry weight yields obtained with the different nitrogen sources under conditions of limiting phosphate, it can be calculated that 2 g of Casamino Acids (lot 479358) contains 6.5 mg (0.325%) of phosphate.

The present study was undertaken to determine the levels of various elements in three lots of Casamino Acids and to determine the degree of correlation between a chemical analysis and the Catenaria bioassay for phosphate. A recent study (7) has provided more complete information on the amino acid composition of these samples of Casamino Acids.

Three methods of analysis were utilized. Phosphate concentrations were determined by the method of Shapiro and Brannock as modified by Boltz and Lueck (1). X-ray fluorescence analyses were done with a Philips PW1212-24 channel automatic X-ray spectrometer calibrated with United States Geological Survey Standards. Atomic absorption data were obtained with a Perkin-Elmer model 303 spectrometer calibrated with Harleco Atomic Absorption Standards. Some analyses were done by Schwarzkopf Microanalytical Laboratory (Woodside, N.Y.). The values given are based upon single determinations unless otherwise stated.

The results (Table 1) indicate a close correlation between the level of phosphate as determined by the Catenaria bioassay (0.325%) and by chemical analysis (0.341%). The acid hydrolysis of casein yields o-phosphorylthreonine (8), and it would be of interest to determine if Catenaria can utilize this source of phosphate or if nonutilization is in part responsible for the difference between the two phosphate values. Although modification of the initial medium and additional replicates might increase the sensitivity of the bioassay, the procedure is too cumbersome to be of practical value. The casein in milk is present primarily as calcium caseinate (8), and, therefore, the high level of calcium (0.043%; 11 mm) is not surprising. Because no growth of Catenaria was obtained on a medium containing Casamino...
Acids but without added calcium or magnesium, these two divalent cations must be chelated by amino acids or otherwise bound and unavailable. This isolate of Catenaria is unable to compete with ethylenediaminetetraacetic acid for calcium and magnesium (6). The very high levels of sodium and chlorine are the result of the neutralization of the hydrochloride-hydrolysate with NaOH. The differences between the sodium and chlorine levels are due to the higher initial level of chlorine in milk (8). The minor components are present at low levels, most below 10 μg/g. The vanadium level appears to be the most variable.

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