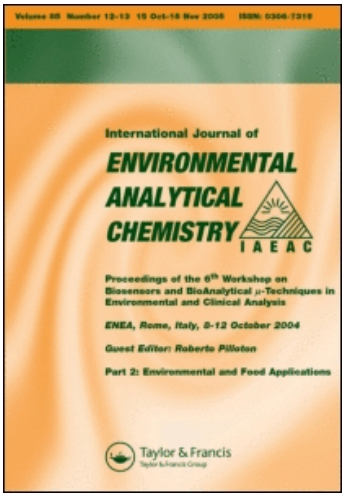

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HPLC Determination of α-Amino Acids in Phytoplanktonic Marine Cells

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ANALYTICAL DATA

HPLC DETERMINATION OF α-AMINO ACIDS IN PHYTOPLANKTONIC MARINE CELLS*

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A procedure for the quantitative determination of 17 amino acids in a marine matrix using HPLC is reported. Pre-column derivatization with *o*-phthalaldehyde, separation on C_{18}-bonded silica with phosphate buffer (pH 7.2)—acetonitrile as eluent and fluorescence detection have been used. The good variation coefficient (average 2% with working curves in real matrix) and the low detection limit (1–5 fmoles) make the procedure suitable for the determination of total or free amino acids in matrix cultures.

KEY WORDS: HPLC, amino acids, phytoplanktonic cells.

INTRODUCTION

Today, there are only a few studies about the amino acid composition of marine phytoplankton species or the marine suspended particulate matter of which they form a part (see, e.g., references 1 and 2). These studies are important because the amino acids of the proteic material of the planktonic population play an important role as energy vehicle and also with regard to the productive power of sea ecosystems. The introduction of RP–HPLC with precolumn derivatization and fluorescence detection simplified this kind of research, although, as emphasized by Mopper *et al.*,[3] many experimental problems still exist.

The aim of this work has been to improve a chromatographic procedure that was already used for other matrices[4] and that, compared with the procedure used up till now for particulate matter,[1,5] uses another organic modifier and affords the advantage of using a more dilute phosphate buffer, which reduces precipitation problems in the analytical column.

EXPERIMENTAL

Instrumentation

The HPLC system was a Bruker Quaternary Gradient LC 2300. The detector was a Bruker LC 315 fluorescence monitor with diffraction grating monochromators, a

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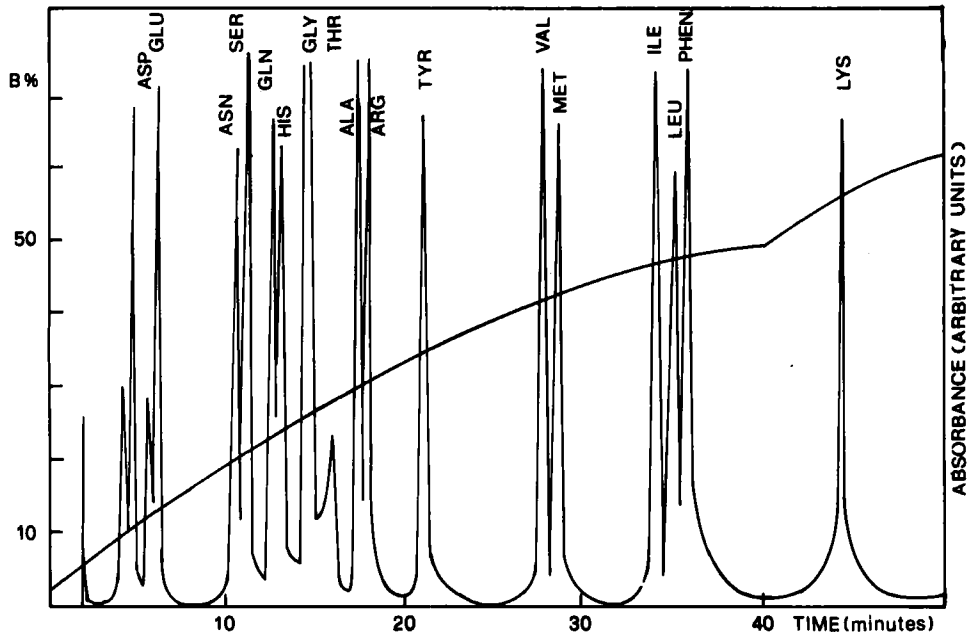

Figure 1 Chromatogram of a standard solution of amino acids (100 pmoles). Gradient run. 250 mm × 4 mm i.d. column packed with 5 μm C_{18}-bonded silica. Mobile phase: (A) 15 mM sodium phosphate, pH 7.2; (B) 45% buffer A and 55% acetonitrile.

Xenon lamp and a 12 μl quartz flow cell. We used 334 and 447 nm as excitation and emission wavelength, respectively. The data system was a LC-21 Epsom microcomputer Q × 10 with a Fx-85 printer. The buffers used were: buffer A (15 mM sodium phosphate, pH 7.2) and buffer B (mixture of 45% buffer A and 55% acetonitrile). The eluents were filtered through 45 μm filters.

The optimized HPLC separation employs an exponential gradient, as shown in Figure 1.

Derivatization

50 mg *o*-phthalaldehyde (OPA) were dissolved in 4.5 ml methanol, and 500 μl 0.025 mM borax buffer adjusted to pH 9.5 with 1 M NaOH, and 50 μl of a solution of β-mercaptoethanol (99%, w/w) were added. 500 μl of a diluted solution of amino acids, 500 μl of borax buffer (pH 9.5), 250 μl of derivatization reagent and 1250 μl of methanol were reacted at room temperature for exactly 3 min.

Hydrolysis

The phytoplankton cells were cultured under bacteriostatic conditions in an erlenmeyer. The medium was sea water filtered over a 0.22 μm Sartorius filter and enriched with nutrients and vitamins (enrichment f/2 of Guillard and Ryter[10] at 1% for nutrients). All cultures were kept at 16 °C and exposed to continuous light

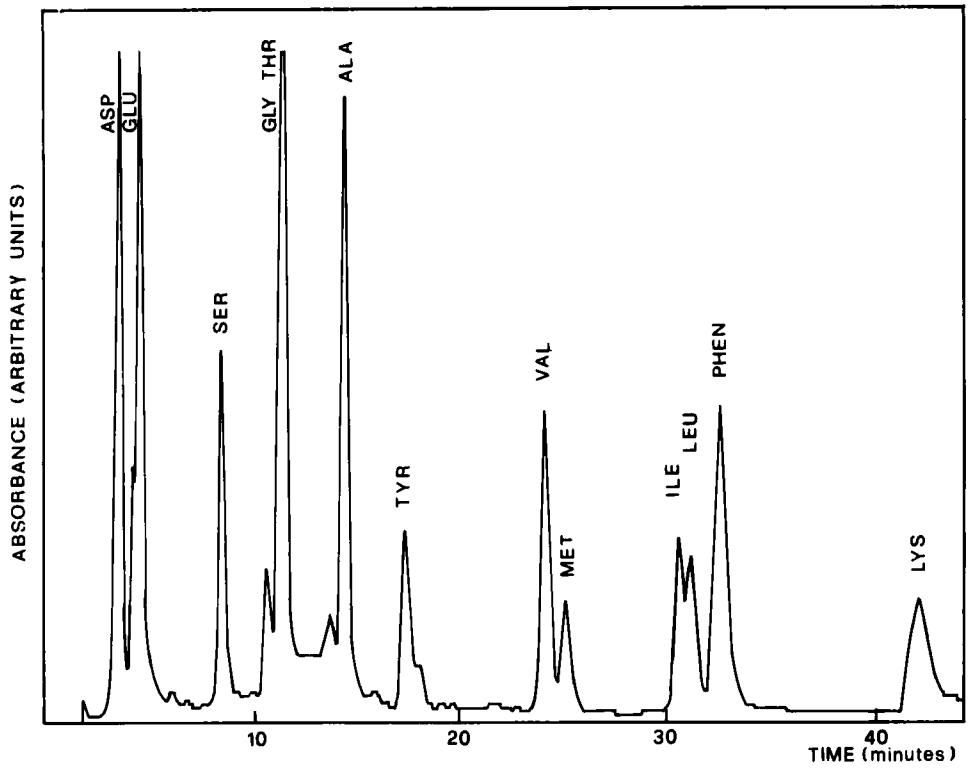

Figure 2 Chromatogram obtained after hydrolysis of a sample of *R. baltica* cells.

(250–280 μE m^{-2} s^{-1}) in the exponential growing phase with aeration. From the onset of the stationary phase the cultures were kept in the dark without aeration, in order to accelerate the decomposition.

5-ml aliquots of culture were filtered with weak suction over Whatman GF/C filters (previously calcined at 500 °C for 30 min). The filters were kept at −60 °C, until the hydrolysis was carried out under nitrogen in 6 M HCl for 22 h at 100 °C.

Analysis

We used the working method, subjecting both the standards and the samples to acid hydrolysis. This is important because of the serine and threonine losses which (can) occur during acid hydrolysis (cf. reference 11).

The detection limits for the amino acids varied from 1 to 5 fmoles; the RSD was on the order of 2% ($n=5$; 20 fmole level, equivalent to a 1 nM concentration in the injected sample).

Application to Analysis of Phytoplankton Cells

The procedure has been applied to cell samples of monospecific cultures of *N. closterium* and *R. baltica*. In Figure 2 we show a typical example of a chromato-

Table 1 Hydrolyzed amino acid content (fmoles/phytoplankton cell) and percentages and total content for *N. closterium* and *R. baltica*

Amino acids	N. closterium				R. baltica			
	I (*n*=6)	%	*F* (*n*=3)	%	*I* (*n*=10)	%	*F* (*n*=6)	%
Asp	5.9	1.1	6.6	2.4	20	4.3	7.1	2.8
Glu	43	7.8	39	14	13	2.8	16	6.2
Ser	38	7.0	7.4	2.7	26	5.6	21	8.2
His	d.l.	0.5	d.l.	—	10	2.1	d.l.	—
Gly+Thr	40	7.5	31	11	52	11	28	11
Ala	7.2	1.3	7.5	2.7	76	16	27	11
Arg	d.l.	—	d.l.	—	d.l.	—	d.l.	—
Tyr	6.4	1.2	64	23	40	8.6	9.8	3.4
Val	45	8.2	15	5.5	35	7.5	13	5.1
Met	18	3.4	10	3.6	20	4.3	2.5	0.98
Ile	90	17	26	9.5	64	14	57	22.4
Leu	103	19	48	17	75	16	44	17.4
Phe	38	7.0	21	7.7	36	7,7	11	4,2
Lys	107	20	d.l.	—	d.l.	—	d.l.	7.0
Total	543		275		467		238	

I, average value from *n* samples of growing phytoplankton cells.
F, average value from *n* samples of degrading phytoplankton cells.

gram obtained with a real sample. In Table 1 the hydrolyzed amino acid contents and the percentages are given. The values were obtained from the analysis of two phytoplanktonic species in the growth and degradation phases. The total amino acids contents are also given.

The reported results indicate a reduction of the total content of the amino acids from the growth to the degradation phase of about 50% for both phytoplanktonic species. The biggest differences for individual amino acids are observed for Tyr and Lys in *N. closterium* cultures. Obviously the present procedure can be applied to real samples, both for culture experiments and for particulate samples. It is relatively rapid, and has sufficient precision and sensitivity to allow determination of small changes in amino acid composition.

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