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Note

Automated ion-exchange chromatographic analysis of usual and unusual natural polyamines

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For the study of polyamine content and metabolism in *Euglena gracilis* Z[1–3], it was necessary to develop a convenient and rapid method for the determination of usual and unusual polyamines such as *sym*-nor-spermidine, *sym*-nor-spermine, *sym*-homospermidine and aminopropylcadaverine, most of which are present in this algal flagellate[1–5]. The method described here allows the determination of histidine, lysine, ethanolamine, carbamylputrescine, arginine, 1,3-diaminopropane, putrescine, histamine, cadaverine, *sym*-nor-spermidine, spermidine, *sym*-homospermidine, agmatine, aminopropyl cadaverine, *sym*-nor-spermine, spermine and 1,7-diaminoheptane in a single sample and without prior purification of the sample at the picomole level in less than 2 h.

MATERIALS AND METHODS

Sample preparation

Aliquots of 50 ml ($1 \cdot 10^5$–$10 \cdot 10^5$ cells) of *Euglena gracilis* (strain Z), grown as described elsewhere[6], were harvested by centrifugation at 5000 *g* for 15 min and the pellet was re-suspended in 0.5 ml of 5% trichloroacetic acid (TCA) in 0.05 *M* hydrochloric acid. The cells were broken by sonication for 20 sec with an MES ultrasonic cell disruptor. The homogenate was centrifuged at 5000 *g* for 15 min and the pellet was then re-extracted with a further 250 μl of 5% TCA in 0.05 *M* hydrochloric acid and re-centrifuged. The two supernatants were then pooled and a 50-μl sample was used directly for the determination of polyamines.

Chemicals

Amino acids and the usual polyamines used were obtained from Sigma (St. Louis, Mo., U.S.A.). *Sym*-nor-spermidine and *sym*-nor-spermine were purchased from Eastman Organic Chemicals (Rochester, N.Y., U.S.A.). N-3-Aminopropylcadaverine and *sym*-homospermidine were gifts from Professor S. S. Cohen (Stony Brook, N.Y., U.S.A.), Dr. D. E. Worth (Parke, Davis & Co., Ann Arbor, Mich., U.S.A.) and Prof. A. N. Radhakrishnan (Hyderabad, India). *o*-Phthalaldehyde was

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obtained from Fluka (Buchs, Switzerland), and all other chemicals used for the preparation of buffers and reagents were obtained as the highest purity grade from Merck (Darmstadt, G.F.R.). All of the amino acid and polyamine solutions were prepared at a concentration of $6 \cdot 10^{-6}$ *M* in 0.1 *M* hydrochloric acid.

Instrumentation and chromatographic conditions

An amino acid analyser (Liquimat-Labotron; Kontron, Vélizy-Villacoublay, France) equipped with a fluorimeter (Labotron FFM-31) using a 50-μl flow cell was employed. An integrator (ICAP-10; LTT, Saint-Honorine, France) was coupled to the fluorimeter for quantification of the amines by the internal standard method. 1,7-Diaminoheptane was used as the internal standard. The recorder (W+W 600-Tarkan; Kontron) was set at 100 mV for 100% relative fluorescence. Separation of amines was carried out on a 11.5 × 0.45 cm I.D. column of DC-4A cation-exchange resin (Durrum, Palo Alto, Calif., U.S.A.). The composition of the two buffers used was as follows: first buffer (pH 5.60), 0.20 *N* sodium citrate dihydrate–0.30 *N* sodium chloride–4% ethanol; second buffer (pH 5.65), 0.20 *N* sodium citrate dihydrate–2.50 *N* sodium chloride–6% ethanol. The buffers and reagent were prepared as described earlier[7–9]. Two temperatures were used: 61° during the first 48 min and thereafter 78° until the end of the chromatographic run. Flow-rates of 26 ml/h for the buffer and 20 ml/h for the reagent were maintained; the first buffer was run for 44 min, then the

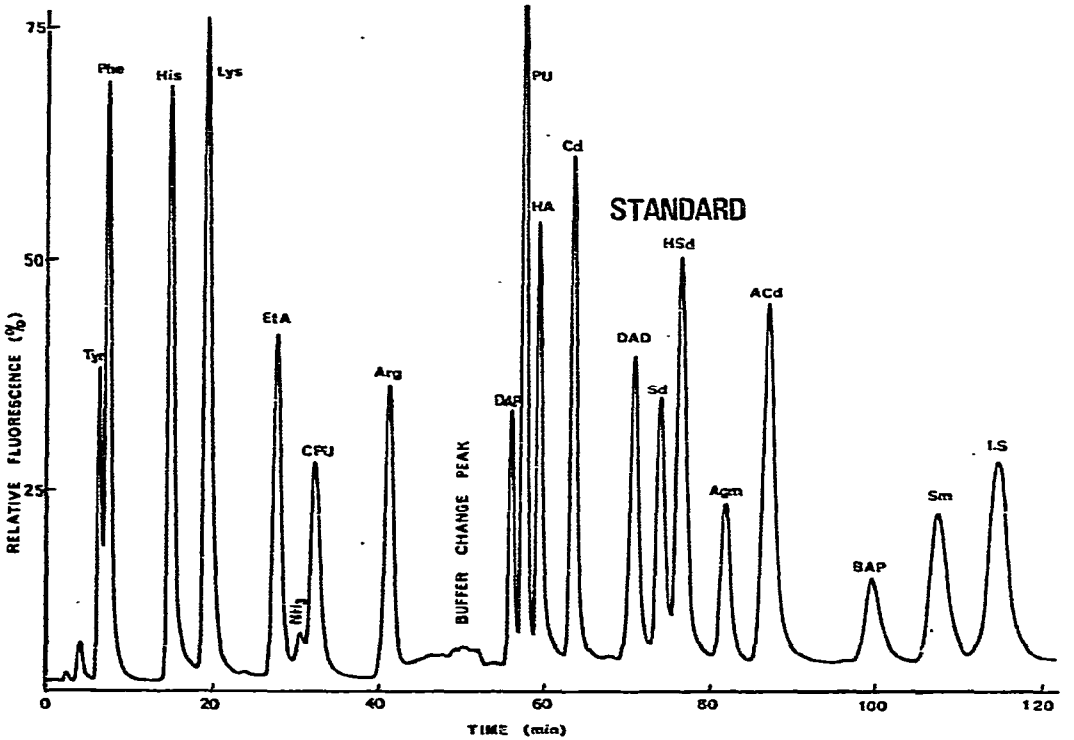

Fig. 1. Chromatographic resolution of a standard mixture of 300 pmole of each of the common basic amino acids and usual and unusual polyamines. Abbreviations as in Table I.

second buffer was run for 68 min. The column was regenerated with 0.2 *M* sodium hydroxide solution containing 250 mg/l of EDTA for 8 min and equilibrated with the first buffer for 30 min.

RESULTS

Fig. 1 shows the elution pattern of a standard mixture (300 pmole of each) of common basic amino acids and usual and unusual polyamines. The components of the mixture, retention times, relative fluorescence peak areas (in arbitrary units) and the coefficient values used for the concentration calculations (KC), using 1,7-diaminoheptane as the internal standard, are presented in Table I. Acidic and neutral amino acids (aspartic acid to phenylalanine) were eluted in a group with the first buffer, and subsequently histidine, lysine, ethanolamine, ammonia, carbamylputrescine and arginine were eluted with good resolution. The second buffer eluted and resolved well the usual and unusual polyamines. The given concentrations of salt and ethanol gave optimal chromatographic resolution. It must be emphasized that the time at which the temperature is changed as well as salt and ethanol concentrations are critical for obtaining good separations of these compounds. Complete analysis was effected in 118 min.

TABLE I

PRECISION AND ACCURACY OF THE METHOD

Compound	*Abbreviation*	*Retention time* (min)*	*Surface area of peaks*	*Constant KC**
Histidine	His	14.7 ± 0.2	86,949	1086
Lysine	Lys	19.2 ± 0.2	103,900	909
Ethanolamine	EtA	27.7 ± 0.1	67,741	1395
Ammonia	NH_3	30.5 ± 0.2	—	—
Carbamylputrescine	CPu	32.1 ± 0.1	67,021	1410
Arginine	Arg	41.1 ± 0.2	64,467	1465
1,3-Diaminopropane	DAP	55.8 ± 0.1	28,465	3320
Putrescine	PU	57.4 ± 0.2	81,197	1182
Histamine	HA	59.3 ± 0.2	72,283	1347
Cadaverine	Cd	63.5 ± 0.1	82,202	1149
3,3′-Diaminodipropylamine (*sym*-nor-spermidine)	DAD	70.7 ± 0.1	72,590	1329
Spermidine	Sd	73.8 ± 0.3	53,158	1777
Sym-homospermidine	HSd	76.3 ± 0.1	91,092	1037
Agmatine	Agm	81.6 ± 0.2	41,229	2472
Aminopropylcadaverine	ACd	86.8 ± 0.1	123,453	833
N,N′-Bisaminopropyl-1,3-propanediamine (*sym*-nor-spermine)	BAP	99.8 ± 0.1	40,014	2546
Spermine	Sm	107.7 ± 0.2	72,596	1329
1,7-Diaminoheptane	I.S.**	114.8 ± 0.2	94,508	

* Average of ten determinations on a mixture containing 300 pmole of each of the products. Quantification was effected with an ICAP 10 integrator coupled to the fluorimeter.

** Internal standard.

Fig. 2 shows the chromatogram obtained from a crude sample of *Euglena gracilis* Z grown in presence of continuous light. Putrescine, *sym*-nor-spermidine, spermidine and *sym*-nor-spermine were found to be the major polyamines present.

The concentrations [expressed as picomoles per cellular volume (μl)] obtained were 257 for putrescine, 179 for *sym*-nor-spermidine, 1160 for spermidine and 1526 for *sym*-nor-spermine. In addition, small amounts of carbamylputrescine, *sym*-homospermidine, 1,3-diaminopropane and spermine were also found.

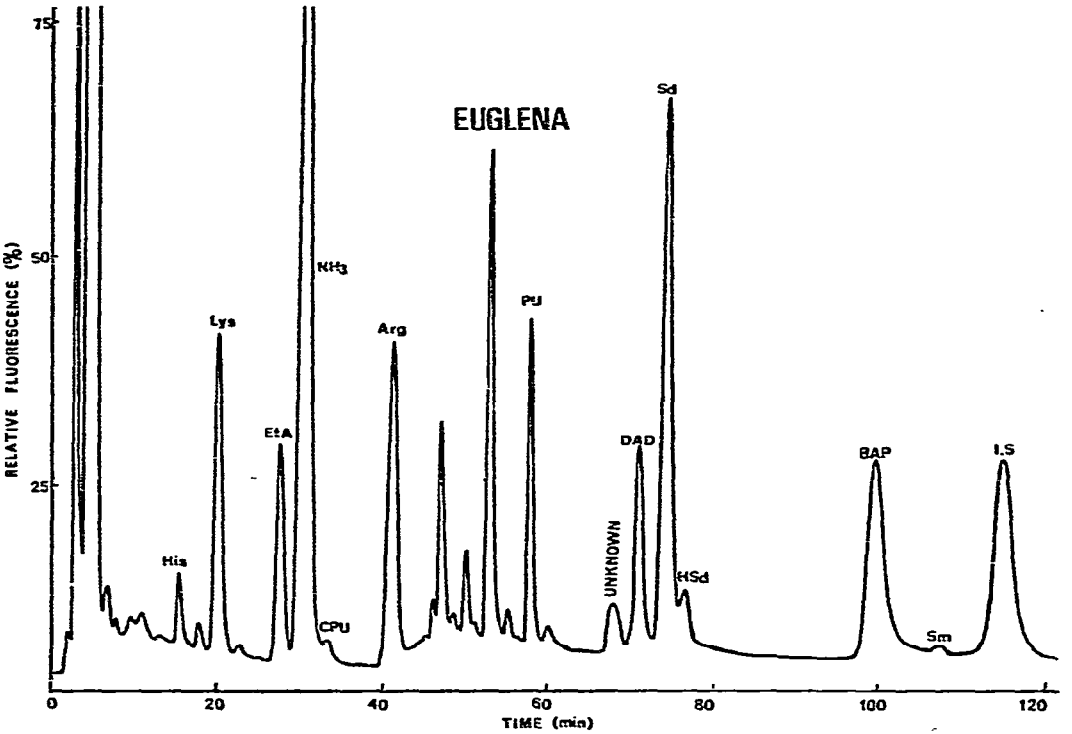

Fig. 2. Chromatogram of a 50-μl crude sample of *Euglena gracilis* Z grown in the presence of continuous light. Abbreviations as in Table I.

DISCUSSION

In this work, the previously described method for the determinations of common basic amino acids, mono-, di- and polyamines and phenolic and indole amines[9] has been further modified so that it could be adapted to the separation of unusual polyamines. The method has been applied to the analysis of these polyamines in crude extracts of *Euglena*. The sensitivity of the method is 1–300 pmole. The presence of large amounts of amino acids in *Euglena* did not interfere with the determination of the amines. The method has proved to be very reliable and the use of an integrator and an internal standard contributed to easier and reproducible quantification. The results presented are averages of ten determinations and the difference between them was never greater than 3%. This method, together with our previously described methods[7–9], would allow the detection of all presently known polyamines, in any kind of crude biological sample.

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