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MEASUREMENT OF FREE AMINO ACIDS IN HUMAN BIOLOGICAL FLUIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Favourable analytical conditions allowing amino acid analysis in biological fluids, acquired from small human biopsy specimens, were achieved by considering various derivatization methods, the mode of detection and the column used. By using *o*-phthalaldehyde–3-mercaptopropionic acid as derivatization agent (high sensitivity and stability) and fluorescence detection (excitation at 330 nm, emission at 450 nm), excellent separation of 26 amino acids was obtained in the lower pmol range (1–10 pmol). The reproducibility of the retention times was better than 1.0% for the majority of amino acids and the results from high-performance liquid chromatography (HPLC), compared favourably with those of conventional amino acid analysis ($r = 0.97$). HPLC technology facilitates amino acid analysis in biopsy specimens of less than 1 mg of tissue.

INTRODUCTION

A quick and routinely manageable method of determining free amino acids has been sought by many investigators. The classical procedure involves separation of amino acids by ion-exchange chromatography, followed by derivatization with ninhydrin[1]. This method, however, suffers from the disadvantage of low mobile phase flow-rate, thereby limiting the rate of sample analyses. A further drawback is the relatively poor sensitivity. Sensitive detection methods are highly desirable considering the progress made in research on amino acid metabolism of individual tissues and cells.

Recent reports emphasize the potential use of high-performance liquid chromatography (HPLC) in amino acid analysis. The application of HPLC reduces the time required for analysis and increases the sensitivity for quantitation of amino acids[2–10]. However, the majority of HPLC applications are restricted to aqueous standard solutions, while the limited data acquired from biological samples frequently yield erroneous concentrations[5–10].

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The purpose of the present study was to develop suitable analytical conditions allowing the determination of free amino acids in plasma as well as in human muscle and liver tissue specimens obtained by needle biopsy. This was achieved by considering various derivatization methods, the mode of detection and by testing different columns. By applying precolumn derivatization with *o*-phthalaldehyde (OPA)–3-mercaptopropionic acid, 28 physiological amino acids could be separated, detected fluorimetrically and quantified. The method facilitates precise measurement of free amino acids in human plasma or tissue in less than 40 min.

MATERIALS AND METHODS

Apparatus

The HPLC system (LKB, Bromma, Sweden) consisted of two Model 2150 pumps, a Model 2152 controller for gradient programming and a Rheodyne injection valve 7125 with a 20-μl filling loop. The following columns were utilized: LiChroCART Superspher CH-8, 4 μm, 250 × 4 mm (Merck, Darmstadt, F.R.G.); Hyperchrome Spherisorb ODS II, 3 μm, 250 × 4.6 mm (Bischoff, Leonberg, F.R.G.). They were maintained at room temperature. The UV detection was usually made at 330 nm with a variable wavelength monitor LKB 2151. Fluorescence was routinely monitored with a Model RF-530 spectromonitor (Shimadzu, Kyoto, Japan). The measurements were made at an excitation wavelength of 330 nm and at an emission wavelength of 445 nm with a 12-μl flow cell and a xenon lamp. Alternatively, a Model FS-970 LC fluorometer (Schoeffel Instruments Corp., Trappenkamp, F.R.G.) equipped with a 5-μl flow cell and a deuterium lamp was used. The excitation monochromator was usually set at 230 nm and a 389-nm emission cut-off filter was used.

Fluorescence characterization of the individual amino acid derivatives was obtained manually with a Model MK II Spectrofluorometer (Farrand, New York, NY, U.S.A.) and by employing an on-line stop-flow procedure with a Model LS-4 spectrometer (Perkin-Elmer, Ueberlingen, F.R.G.) equipped with a xenon lamp (excitation wavelength 330 nm, slit 5 nm; emission wavelength 445 nm, slit 10 nm) and a 3-μl flow cell. UV spectra were obtained with a Uvicon 820 spectrophotometer equipped with an LS Printer 48 and a Recorder 21 (Kontron, Zürich, Switzerland). UV absorption and fluorescence was recorded with a double pen recorder LKB 2210 at a sensitivity of 10 mV and at a speed of 30 cm/h.

Continuous on-line quantitation of the HPLC results was obtained with a Model Chromatopac C-R2AX and C-R1B data processor (Shimadzu).

Reagents

Ultra pure water generated with an Elgastat Spectrum water purification system, including reverse osmosis, activated carbon and nuclear grade ionization cartridges (Elga, Lane End, U.K.), was always used in the preparation of buffers.

The chemicals utilized were of analytical grade and the solvents of chromatographic grade. Sodium phosphate, boric acid, sodium hydroxide, methanol, acetonitrile (ACN), ethanethiol (ET) and 5-sulphosalicylic acid (SSA) were purchased from Merck. The internal standard norvaline (Sigma, St. Louis, MO, U.S.A.) was dissolved in a SSA solution yielding a final concentration of 100 nmol/ml. 3-Mercaptopropionic acid (3-MPA) was obtained from Fluka (Buchs, Switzerland); 2-mercaptoethanol (2-ME) and OPA from Serva (Heidelberg, F.R.G.).

An amino acid standard containing 17 amino acids, representative of a protein hydrolysate, was obtained from LKB Biochrom (Cambridge, U.K.). A second amino acid standard containing 28 amino acids, representative of a physiological experiment, was prepared by adding crystalline α-aminoadipic acid, α-aminobutyric acid, asparagine, glutamine, citrulline, carnosine, 1-methylhistidine, 3-methylhistidine, ornithine, taurine and norvaline to the commercial LKB standard. The individual crystalline amino acids were obtained from Serva. Amino acid standard solutions were prepared in water and were kept at −70°C until analyzed.

Preparation of the OPA/mercaptan derivatization agents

A 50-mg amount of OPA was dissolved in 4.5 ml methanol. A 50-μl volume of ET, 2-ME or 3-MPA was added together with 0.5 ml of 1.0 *M* sodium borate (pH 9.5)[5]. The reagent mixture was kept in the dark at 4°C. Fresh mixtures were prepared each week.

Preparation of biological samples

Plasma. Heparinized blood was obtained from seven healthy young males and centrifuged at 2000 *g* for 15 min. The plasma was then immediately deproteinized with SSA (30 mg SSA per ml plasma). The protein-free filtrate was processed as described earlier[11] and stored at −70°C until analyzed.

Muscle. Samples (*ca.* 10 mg) from intensive care patients were obtained by the percutaneous needle biopsy technique[12], homogenized in 0.5 ml of 4% SSA and treated as described[11,13].

Liver. Biopsy specimens (*ca.* 10 mg) were obtained from patients undergoing gastro-intestinal surgery. The samples were transferred, as rapidly as possible (20 sec), into liquid nitrogen and then stored at −70°C until lyophilized and subsequently pulverized. Visible connective tissue and fat were removed[14,15]. Liver free amino acids were extracted with 4% SSA (4 mg SSA per mg dry liver solid) and the protein-free filtrate was treated as described for muscle[11,13].

Derivatization procedure

A two-step procedure (Table I) was employed for biological samples, using chemicals of analytical grade. This procedure diminished the occurrence of interfering OPA-adducts, which seriously disturbed the baseline at the applied range of sensitivity. Direct derivatization, however, is possible when applying ultra pure chemicals.

Gradient

The program applied and the two helium-conditioned solvents used are given in Table II. The flow-rate was maintained at 1.2 ml/min throughout.

Conventional ion-exchange amino acid analysis

The HPLC results were compared with those acquired using an LC-5000 amino acid analyzer (Biotronic, München, F.R.G.).

RESULTS AND DISCUSSION

In the present work the main efforts were directed to develop a suitable method

TABLE I

TWO-STEP DERIVATIZATION PROCEDURE WITH OPA/3-MPA FOR AMINO ACID STANDARDS AND BIOLOGICAL FLUIDS, RESPECTIVELY

	Component	*Standard mixture (µl)*	*Sample mixture (µl)*
I.	Standard	5	–
	Sample	–	50
	Methanol	500	500
	Sodium borate	445	400
	OPA/3-MPA	50	50
	Total mixture volume (µl)	1000	1000
II.	After a 5-min incubation, 495 µl and 450 µl sodium phosphate buffer (12.5 m*M*, pH 7.2) were added to a 5-µl standard mixture and to a 50-µl sample mixture, respectively. After thorough mixing, 20 µl were then injected.		

TABLE II

CHROMATOGRAPHIC GRADIENT CONDITIONS FOR HPLC ANALYSIS OF OPA/3-MPA-AMINO ACID DERIVATIVES

Buffers: A, 12.5 m*M*, sodium phosphate, pH 7.2; B, 50% acetonitrile in 12.5 m*M* sodium phosphate, pH 7.2.

Duration (min)	*From (% buffer A/B)*	*To (% buffer A/B)*
0–4	100/0	94/6
4–6	94/6	92/8
6–9	92/8	92/8
9–17	92/8	84/16
17–23	84/16	80/20
23–34	80/20	67/33
34–42	67/33	50/50
42–45	50/50	0/100
45–47	0/100	0/100
47–50	0/100	100/0

for optimum separation of free amino acids in physiological fluids. One of the most essential tasks in this respect is to define the most favourable conditions as far as the derivatization procedure (reaction time and reaction composition) is concerned. In the majority of prior applications, 2-ME[3,4,6,7,9,10,16–23] or occasionally ET[2,5,8,24] was utilized. In a recent kinetic investigation 3-MPA was suggested as the most suitable reagent, revealing the best fluorescence response and stability[25].

We measured excitation and emission maxima of the OPA/3-MPA derivatives of 21 amino acids. The mean excitation maximum was 334.5 ± 1.5 nm (S.D.), range 332–338 nm. Lysine was the only exception, exhibiting an excitation maximum at 311 nm; hence it was excluded from the calculation. The mean emission maximum was 445 ± 2.06 nm (S.D.), with a range of 440–449 nm. The above fluorescence characteristics were fully confirmed with on-line stop-flow measurements.

The stability of the OPA-amino acid adducts was investigated in three alternative experiments applying ET, 2-ME or 3-MPA in the reagent mixture. The results are shown in Fig. 1 as graphs relating the UV absorbance of the OPA-amino acid derivatives to the reaction time and UV response for the three different mercaptan reagents. Maximum UV intensity was achieved after 130, 240 and 400 sec when employing ET, 2-ME and 3-MPA, respectively. The best UV response and stability was obtained with OPA/3-MPA. When employing 2-ME, however, the degradation of OPA-amino acids is apparent.

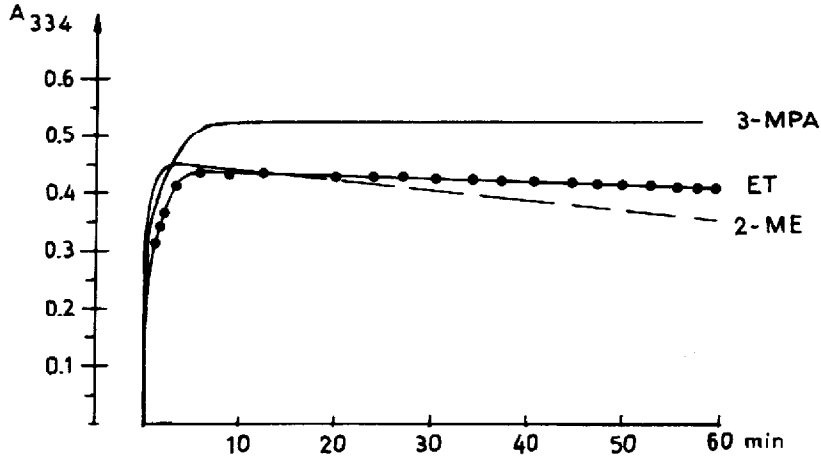

Fig. 1. The stability of the OPA-amino acid adducts illustrated as the UV response *versus* reaction time for the three mercapto reagents tested. OPA/ET, OPA/2-ME or OPA/3-MPA are added to the methanol–sodium borate buffer containing the 17 amino acid standard (each amino acid was 2.5 nmol/ml). UV absorbance was monitored at 334 nm.

Indeed, simple measurement of UV absorbance is inconclusive, especially for a mixture of amino acids. In preliminary studies we examined the individual fluorescence responses of 26 OPA/3-MPA amino acid derivatives and found maximum fluorescence emission 2.5 min after the onset of reaction. This observation is in perfect agreement with that reported by Kucera and Umagat[25]. The calculated apparent fluorescence constants, FK′, and the relative fluorescence for each amino acid are listed in Table III. Each apparent constant was calculated by considering the respective area (μV output unit) as an expression of the fluorescence yield per 1 pmol of the corresponding individual amino acid derivative. The relative fluorescence was calculated by taking phenylalanine as reference amino acid (Phe = 1.00). Carnosine reveals the highest fluorescence yield, whereas lysine and ornithine showed the lowest relative fluorescence.

However, it must be noted that the relative fluorescence response of OPA/3-MPA adducts, as calculated in Table III, differs considerably from the response factors calculated by other investigators using ET or 2-ME[2,4,16]. It might be concluded that relative response factors should be calculated for each particular experimental condition, since changes in system, derivatization agent, pH, gradient profile, etc., will alter these values. Nevertheless, in the present study, distinct excitation and emission maxima for all amino acids (except for lysine) were observed, and the relative

TABLE III

CALCULATED APPARENT FLUORESCENCE CONSTANT (FK') AND THE RELATIVE FLUORESCENCE FOR 26 OPA/3-MPA-AMINO ACID DERIVATIVES

Amino acid	*FK' (area/pmol · 10^3)*	*Relative fluorescence (phenylalanine = 1.0)*
Aspartic acid	13.5	0.75
Glutamic acid	14.0	0.78
α-Aminoadipic acid	16.0	0.89
Asparagine	20.3	1.13
Serine	16.5	0.92
Glutamine	18.0	1.00
Glycine	17.0	0.94
Threonine	15.0	0.83
Histidine	8.5	0.47
Citrulline	17.0	0.94
Alanine	15.5	0.86
1-Methylhistidine	17.8	0.99
Carnosine	22.0	1.22
3-Methylhistidine	19.5	1.08
Arginine	16.5	0.92
Taurine	8.5	0.47
α-Aminobutyric acid	15.0	0.83
Tyrosine	15.0	0.83
Valine	16.3	0.91
Methionine	16.5	0.92
Norvaline	21.0	1.17
Isoleucine	18.3	1.02
Phenylalanine	18.0	1.00
Leucine	18.3	1.02
Ornithine	3.3	0.18
Lysine	2.9	0.16

fluorescence responses calculated (Table III) were in accord with values obtained when using OPA/2-ME[4].

Two amino acid standards containing 17 amino acids (representative of a protein hydrolysate) and 28 amino acids (representative of a physiological sample) revealed excellent separation (Figs. 2 and 3), but cysteine and proline were not detectable. The favourable resolution achieved was irrespective of the type of fluorimeter used. These results encouraged us to use an instrument equipped with a xenon lamp, 12-μl flow cell and preset excitation and emission wavelengths. By using an alternative instrument with a deuterium lamp, 5-μl flow cell and cut-off filter, there was no appreciable improvement in resolution (Fig. 2), while the sensitivity was about 3–4 times higher.

As pointed out by Lindroth and Mopper[4], an excess of reagent is necessary to facilitate linearity. Accordingly, in the present investigation an excess of the OPA/3-MPA was used as outlined under Methods and in Table I. The linearity was then calculated at various concentrations (125–1000 pmol/ml) of 21 amino acids by considering the relative fluorescence (area) of each individual amino acid. As shown in Fig. 4, the linearity was good for all amino acids analyzed, irrespective of fluorescence yield.

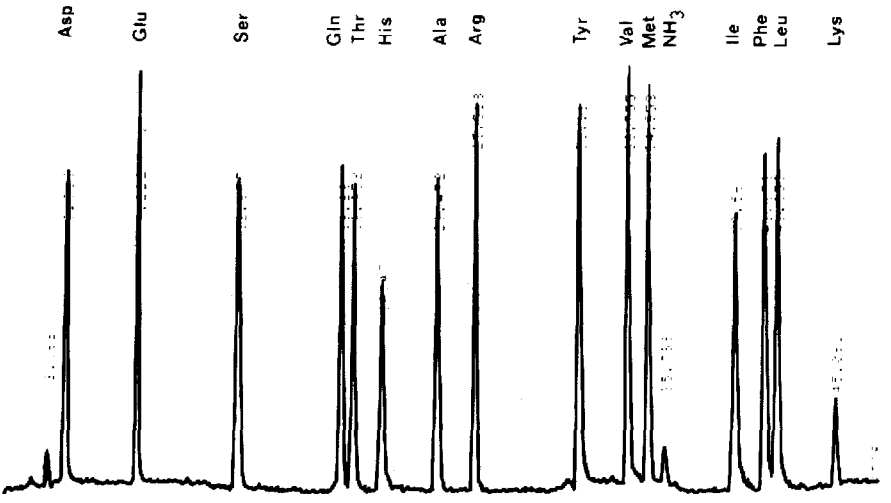

Fig. 2. Chromatogram of an amino acid standard containing seventeen amino acids. The separation was made on a Spherisorb ODS II, 3-μm column. Fluorescence intensity was monitored at an excitation of 230 nm and an emission of 389 nm (cut-off filter) (Schoeffel, FS-970). The sensitivity was set at 0.01 range unit. Each amino acid had a concentration of 125 pmol/ml. Twenty microlitres of the standard were injected (2.5 pmol of each amino acid).

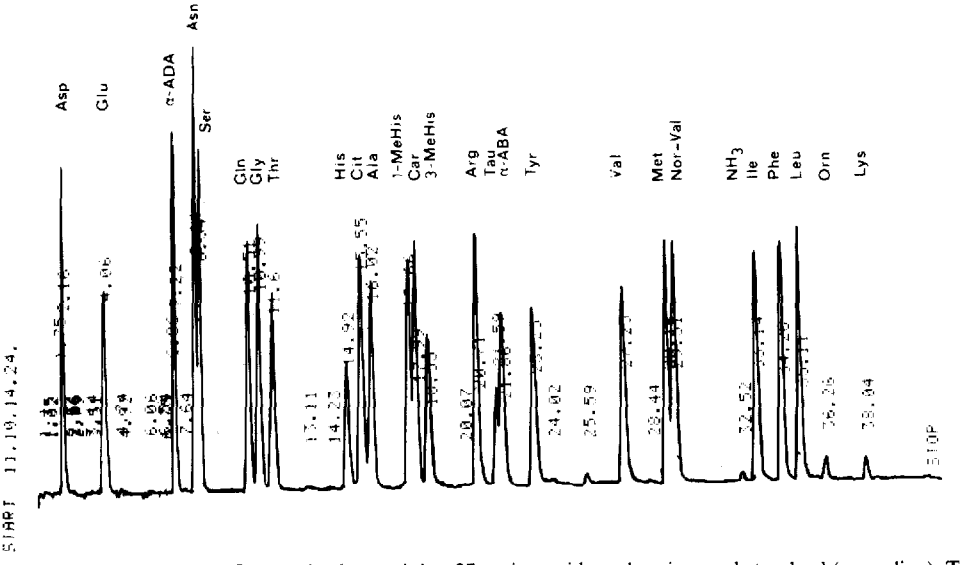

Fig. 3. Chromatogram of a standard containing 27 amino acids and an internal standard (norvaline). The separation was made on a Superspher CH-8, 4-μm column. Fluorescence intensity was monitored at an excitation of 330 nm and an emission of 445 nm (Shimadzu, RF-530). The sensitivity was set at 2 range units. Each amino acid had a concentration of 500 pmol/ml. Twenty microlitres of the standard were injected (10 pmol of each amino acid).

The objective of the present work was focused on the determination of free amino acids in physiological fluids. In Fig. 5, typical chromatograms of plasma (a), muscle (b) and liver (c) tissues are given, showing that 26 free amino acids present in these biological materials could be satisfactorily separated, identified and subse-

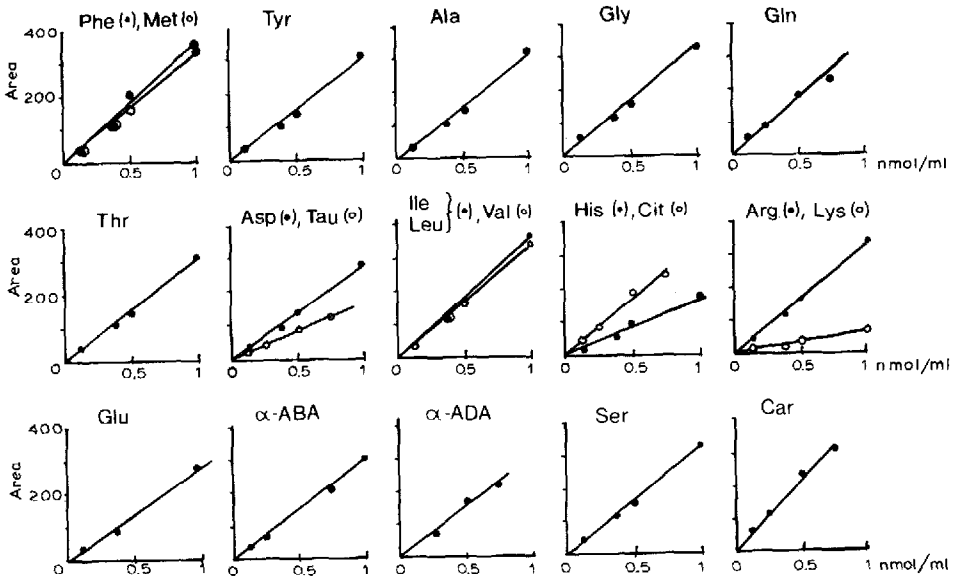

Fig. 4. The linearity obtained for 21 amino acids in the concentration range of 0.125–1.0 nmol/ml (injection volume 20 μl).

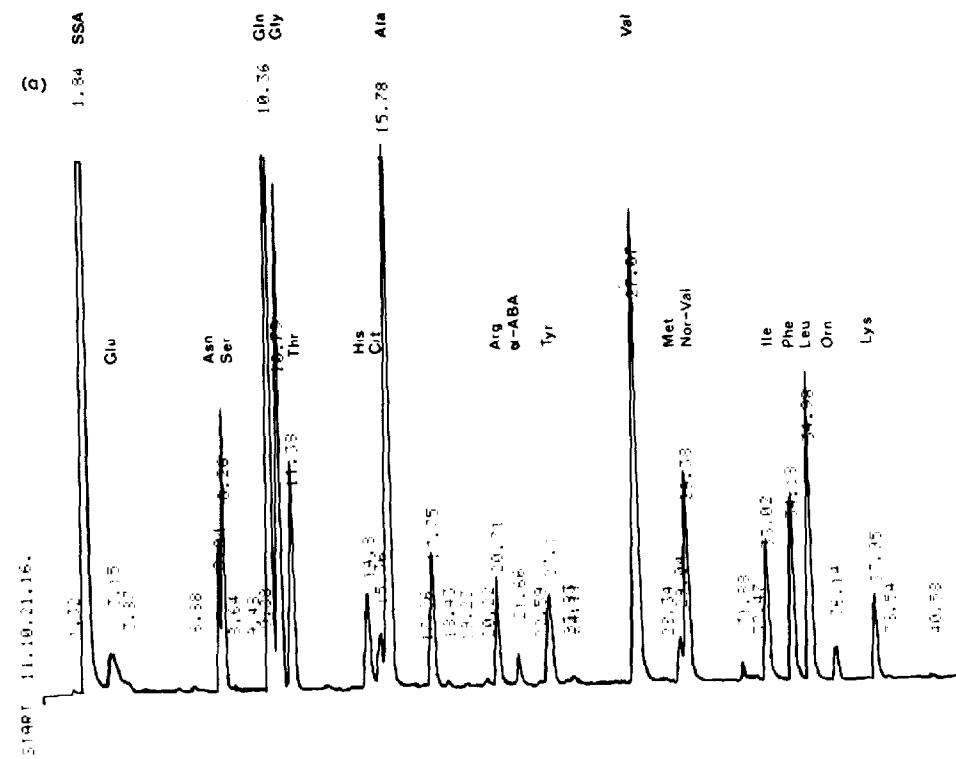

Fig. 5.

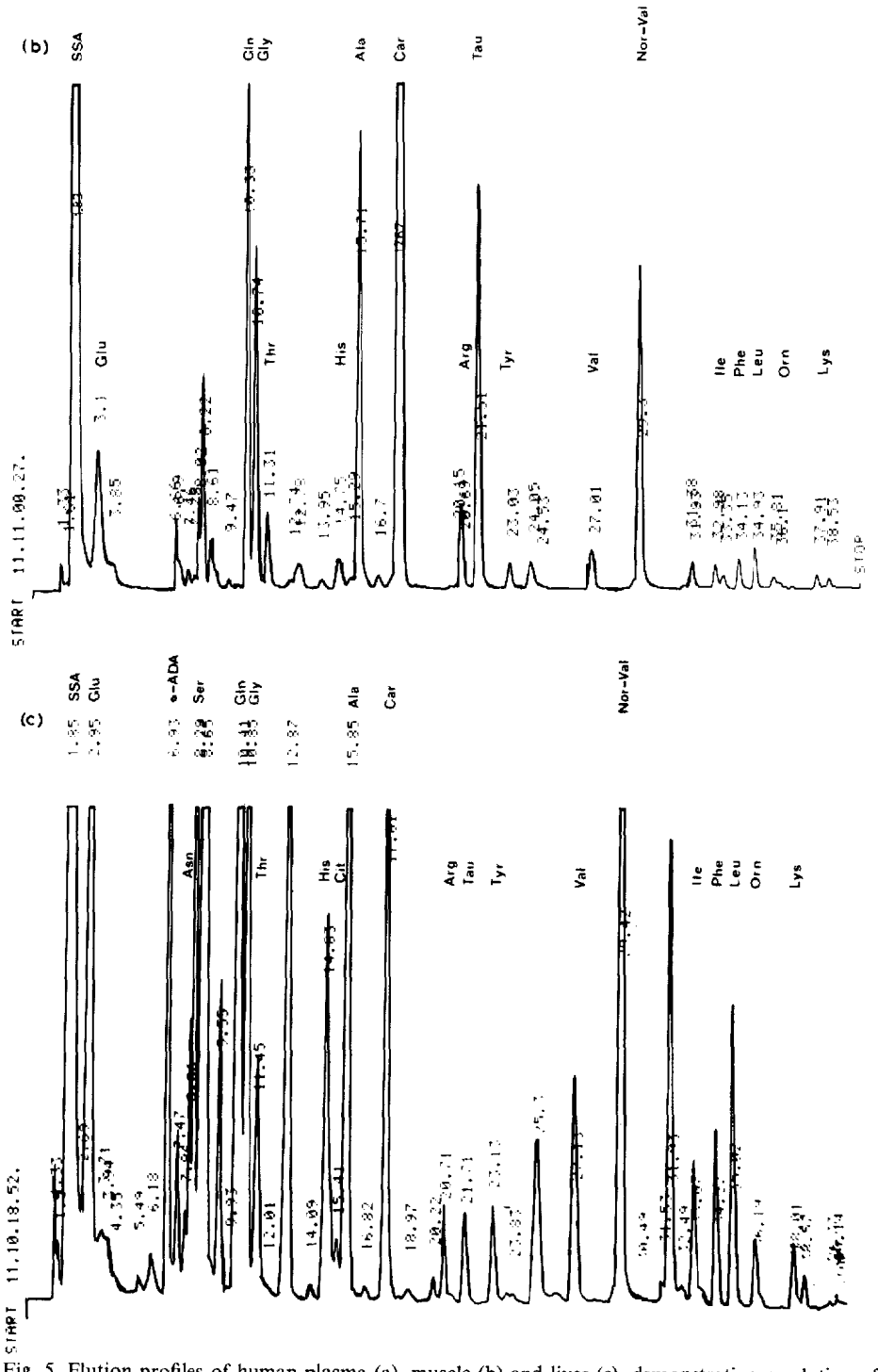

Fig. 5. Elution profiles of human plasma (a), muscle (b) and liver (c), demonstrating resolution of free amino acids by HPLC. Fluorescence intensity was monitored with excitation at 330 nm and emission at 445 nm (Shimadzu, RF-530).

quently quantified with the aid of standards (Figs. 2 and 3). Free amino acid concentrations in tissue as determined by HPLC in the present work were in good agreement with results reported earlier obtained by using automated ion-exchange amino acid analyzers[11,13,26–28].

The mean free amino acid concentrations in plasma from seven healthy subjects are presented in Table IV. The results are essentially in agreement with normal values[11,13,28,29]. A comparison between HPLC amino acid results and those acquired from the conventional amino acid analyzer for the same samples is included in Table IV and illustrated in Fig. 6. The HPLC results compared favourably with those obtained with the conventional amino acid analyzer. The actual correlation between the two methods was highly significant ($r = 0.97$, $p < 0.001$) in 120 comparative analyses. It is worth noting that although the same concentration was observed for the majority of amino acids with either method, the levels of 3-methylhistidine, α-aminoadipic acid and α-aminobutyric acid were found to be lower with HPLC. Presumably the true concentrations of these amino acids were approached more closely with HPLC than with the amino acid analyzer, since the separations of α-aminoadipic acid and α-aminobutyric acid are reported to be poor when using ion-exchange chro-

TABLE IV

FREE AMINO ACID CONCENTRATIONS IN PLASMA FROM SEVEN HEALTHY SUBJECTS AS DETERMINED BY HPLC AND AUTOMATED ION-EXCHANGE CHROMATOGRAPHY (μmol/l; MEAN ± S.E.M.)

Results are given in μmol/l as mean ± S.E.M.

Amino acid	*HPLC*	*Ion exchange*
Glutamic acid (1)	21.4 ± 8.53	24.8 ± 5.44
α-Aminoadipic acid (2)	4.0 ± 0.53	14.3*
Asparagine (3)	47.1 ± 2.42	51.2 ± 5.99
Serine (4)	138.5 ± 9.38	119.9 ± 10.64
Glutamine (5)	577.0 ± 24.36	574.5 ± 31.89
Glycine (6)	264.3 ± 23.84	254.4 ± 24.77
Threonine (7)	139.6 ± 8.21	135.3 ± 9.65
Histidine (8)	94.0 ± 3.28	87.27 ± 4.4
Citrulline (9)	30.8 ± 1.39	30.5 ± 1.84
Alanine (10)	370.6 ± 18.4	411.6 ± 28.4
1-Methylhistidine (11)	37.0 ± 8.21	28.5 ± 5.00
3-Methylhistidine (12)	13.0 ± 2.77	19.95 ± 1.53
Arginine (13)	84.5 ± 7.24	84.8 ± 7.18
Taurine (14)	88.9*	66.4 ± 6.0
α-Aminobutyric acid (15)	25.5 ± 3.36	33.2 ± 2.97
Tyrosine (16)	68.0 ± 3.64	65.8 ± 3.78
Valine (17)	286.7 ± 16.42	258.3 ± 14.11
Methionine (18)	24.8 ± 1.65	27.12 ± 2.63
Isoleucine (19)	78.6 ± 3.68	75.00 ± 3.85
Phenylalanine (20)	98.3 ± 4.95	74.1 ± 2.4
Leucine (21)	141.6 ± 4.87	138.5 ± 5.6
Ornithine (22)	86.6 ± 4.35	79.1 ± 5.7
Lysine (23)	245.0 ± 7.04	166.7 ± 6.68

* Derived from two determinations only.

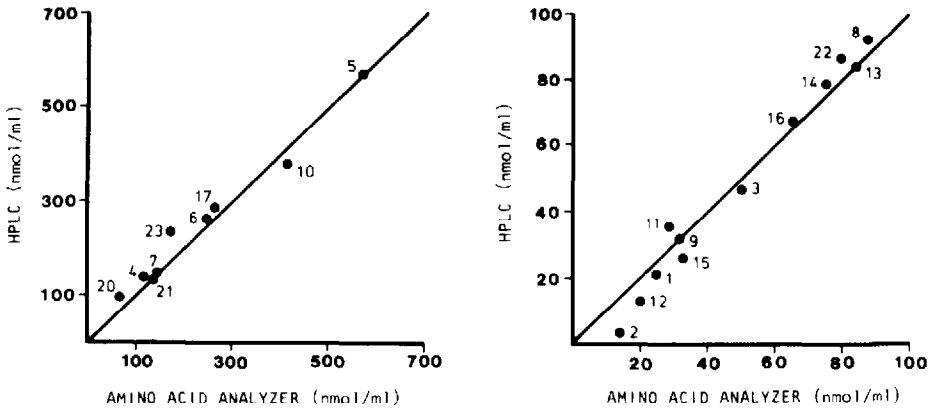

Fig. 6. Comparison between results derived from HPLC and a conventional amino acid analyzer. The numbers indicated the amino acids in Table IV.

matography[29,30]. On the other hand, excellent quantitative results with HPLC for 3-MeHis are now repeatedly achieved[6,31].

Under the present analytical conditions a full physiological analysis took about 40 min and, as recorded in Table V, gave reproducible separations. The reproducibility of the retention times of the 17 amino acid standard and physiological analyses are given in Table V. The reproducibility was calculated as the deviation in a single

TABLE V

REPRODUCIBILITY OF THE RETENTION TIMES (t) OF THE 17 AMINO ACID STANDARD (PROLINE AND CYSTEINE ARE NOT DETECTABLE) IN FIFTEEN ANALYSES AND THOSE OF HUMAN PLASMA IN TEN ANALYSES

Reproducibility is calculated as the deviation of a single determination from the mean ($S.D._d$) and also expressed by the coefficient of variation (CV), see text.

Amino acid	*Standard*			*Plasma*		
	t	$S.D._d$	*CV (%)*	t	$S.D._d$	*CV (%)*
Aspartic acid*	2.18	0.05	2.3	–	–	–
Glutamic acid	4.06	0.17	4.2	3.14	0.12	3.8
Serine	8.74	0.31	3.6	8.49	0.05	0.6
Glycine	12.50	0.23	1.8	11.54	0.11	0.9
Threonine	13.34	0.16	1.2	12.37	0.11	0.9
Histidine	16.23	0.1	0.7	16.15	0.19	1.2
Alanine	17.81	0.12	0.7	17.1	0.15	0.9
Arginine	22.1	0.23	1.0	22.3	0.28	1.3
Tyrosine	25.23	0.16	0.6	24.98	0.18	0.8
Valine	29.43	0.18	0.6	29.37	0.2	0.7
Methionine	31.04	0.16	0.5	31.35	0.32	1.0
Isoleucine	33.41	0.24	0.7	35.29	0.24	0.7
Phenylalanine	34.3	0.16	0.5	36.45	0.28	0.8
Leucine	35.27	0.12	0.3	37.22	0.22	0.6
Lysine	39.48	0.16	0.4	39.85	0.32	0.8

* Derived from eleven determinations only.

determination from the mean retention time according to the formula $S.D._d = \Sigma(d - \bar{d})^2/(n-1)^{1/2}$, where $d = (x_n - \bar{x})$ in fifteen standard analyses and in ten physiological analyses, respectively. The coefficient of variation (CV) was determined according to the formula $CV = (S.D._d/\bar{x}) \cdot 100$. The average CV for the fifteen amino acids was better than 1.0% and in agreement with other investigations[4,6,7,9] the largest variation was found for aspartic and glutamic acids closest to the injection point.

In biological samples, OPA/3-MPA-aspartic acid is superimposed by the deproteinizing agent SSA. This problem can be overcome by using 2-ME according to a recent report from Hogan *et al.*[7] In this case, however, there may be difficulties due to the continuous degradation of the OPA/2-ME-amino acid[4,15] resulting in an unstable system. Difficulties associated with aspartic acid and ornithine were also contemplated by Fleury and Ashley[8] when using OPA/ET. Turnell and Cooper[6] and Jones and Gilligan[10] used acetonitrile as precipitation reagent and obtained excellent separations of aspartic acid. However, their reported mean value highly exceeded the true physiological concentration, not only for aspartic acid but for the majority of other free amino acids determined. It is thus conceivable that the use of acetonitrile results in proteolysis, thereby erroneously enhanced free amino acid concentrations in biological fluids. In the present study, phenylalanine and tryptophan were eluted together, resulting in erroneously high plasma phenylalanine values (Table IV, Fig. 6). Inferior separations between glycine and threonine[5,7] or asparagine and serine[9] have been reported when using OPA/2-ME, whereas co-elutions of tyrosine and α-aminobutyric acid or glycine and citrulline were noted with OPA/ET[8]. None of these difficulties was observed in the present investigation.

The plasma level of free lysine determined in the present study was within the normal range found by other investigators[6,10]. However, in the majority of investigations the plasma lysine concentrations are overestimated[5,8,9] or underestimated[7]. Thus, quantitation of lysine seems to be a common problem especially in biological fluids, probably because of the poor fluorescence yield (Table III). This difficulty calls for detailed future investigations concerning the kinetics and thermodynamics of this OPA adduct. It is worth noting that addition of BRIJ 35 to the derivatization agent might increase the fluorescence response of lysine[3,10].

As shown in Fig. 5, all free amino acids of common interest, except proline, hydroxyproline and cysteine, could be identified and quantified. Derivatization with OPA cannot be used to detect proline and hydroxyproline[18,32], whereas cysteine is recommended to be detected in the presence of iodoacetic acid[33]. On the other hand, owing to the obvious risk of loosing tyrosine and histidine[33], important tissue amino acids, we relinquished the use of iodoacetic acid in the present work.

The high flexibility of the HPLC technique, as far as choice and variability of chromatographic parameters are concerned, enables the separation and quantification of rare free amino acids in tissue like phosphoserine, phosphoethanolamine, β-alanine, hydroxylysine, γ-aminobutyric acid, etc. However, as mentioned, a sufficient amount of reagent is of special importance when dealing with physiological samples, since reagent-consuming amines, although unidentified, are always present (Fig. 5). When access to ultra pure chemicals is not available, it is highly recommended to dilute physiological samples so as to avoid or diminish baseline disturbances and interferences due to OPA-adducts derived from biological amines[4,32] and from reagent contaminants.

OPA precolumn derivatization and RP-HPLC offers advantages over conventional ion-exchange chromatography and ninhydrin detection when dealing with microsamples. For a satisfactory HPLC analysis, $\leqslant 1$ mg tissue is sufficient instead of $\geqslant 10$ mg for a conventional analysis. Besides the high sensitivity, the considerable reduction of the analysis time (about 1/3 as compared to an amino acid analyzer) is worth noting.

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