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Quantitative analysis of 17 amino acids in the connective tissue of patients with pelvic organ prolapse using capillary electrophoresis–tandem mass spectrometry

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Abstract

The simultaneous determination of 17 amino acids in connective tissue using capillary electrophoresis is described in this study. Separation was carried out on a fused silica capillary column (80 cm × 50 mm i.d.) with 1 M formic acid as the running electrolyte. The detection was conducted on a mass spectrometer by selective reaction monitoring (SRM) mode via an electrospray ionization source. Tissue samples were prepared by reduction and acid hydrolysis to extract amino acids; over 84.3% recovery was seen for all compounds. The method allowed for sensitive, reproducible, and reliable quantification, and all 17 amino acids were separated using this method. Good linearity over the investigated concentration ranges was observed, with values of R higher than 0.993 for all the analytes. Precision and accuracy examined at three concentration levels ranged from 0.2% to 19.5% and 84.1% to 120.0%, respectively. Matrix effects were also tested and ranged from −9.1% to 15.4%. The validated method was applied to the quantitation of 17 amino acids in pelvic connective tissue of pelvic organ prolapsed patients. Methionine, glutamine, and histidine were significantly higher in the experimental patients compared to the controls. This suggests that changes in the amino acid concentrations within the connective tissue could be a factor in the genesis of pelvic organ prolapse. Therefore, this method is potentially applicable for amino acid analysis in tissue, providing a more complete understanding of pelvic organ prolapse.

Keywords: Amino acid; CE/MS; Connective tissue; Quantitative analysis

1. Introduction

Amino acids (AAs) play an important role in maintaining the immune system, and along with their derivatives, regulate metabolic pathways by synthesizing proteins through biological processes [1,2]. Various metabolic and chromosomal disorders, which result from an enzyme deficiency, can be detected by monitoring AAs and organic acids in biological matrices [3–5]. Therefore, quantitative determination of AAs in various biological samples, such as urine, serum, and biological tissues, is widely used in clinical diagnostics. Moreover, simultaneous determination of these AAs in physiological samples may play a critical role in discovering biomarkers for specific disease groups [6].

Several analytical methods have been described for the determination of AAs in clinical practice. Leaving the outdated methods aside, using HPLC-based automated amino acid analyzers is a very straightforward and advanced technique. However, they are currently easily applicable for only a few AAs, and significant time is required for pre- or post-column derivatization, in order to increase the separation efficiency and detection limit [7,8]. Despite having high resolving power and efficiency in the case of gas chromatography/mass spectrometry (GC/MS) and the ability to separate many metabolites with a good peak shape using HPLC, this technique requires significant amounts of sample. Thus, Soga T and Monton MRN

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have suggested that capillary electrophoresis/mass spectrometry (CE/MS) is a powerful technique in metabolome analysis and biomarker discovery, which not only has excellent resolving power but also requires only minute amounts of sample [9].

The major advantages of coupling CE with MS are the extensively high resolution, good separation speed, selectivity, and sensitivity; essentially any kind of charged compound can be detected [10]. Due to their charged nature in solution, AAs were the first compound to be investigated by CE. Lu et al. first demonstrated the possibility of CE–MS for the analysis of AAs [11,12].

CE/MS and CZE (capillary zone electrophoresis)/MS have been successfully applied to determine AAs in the food industry [13–15]. AAs have also been analyzed on other biological samples, such as amniotic fluids, blood, and human vitreous humor using various analytical approaches of CE, such as contactless conductivity detection, CE, or coupling CE with MS, respectively [16–18]. Soga and co-workers have established a feasible CE/MS method to analyze AAs in urine and soy sauce [19,20]. In a recent study of AA profiling in urine, an optimized method of CE/time of flight (TOF)-MS has been developed. All the aforementioned methods for AA analysis addressed a number of important technical issues [21].

This study focuses on the quantitative analysis of AAs in pelvic connective tissue since the component changes of AAs in this tissue could be an important factor in the genesis of pelvic organ prolapse (POP).

POP is an aging problem, which is the second most common problem in postmenopausal women. The natural history and mechanism of POP is not yet well understood. However, it is thought that a collagen abnormality is one of the contributing factors [22,23]. Various investigators have already demonstrated decreased collagen content and altered morphological features in the pelvic support tissue in women with POP [24–26]. Collagen is composed of several AAs, hydroxyproline, proline, glycine, and hydroxylysine, and changes of morphological features in pelvic tissue are very closely related to the change of AAs. However, the method of quantification for the determination of AAs in tissue has not been fully reported yet. A few studies have been found on tissue AA analysis with CE/MS. Liu et al. described a method to detect derivatized AA enantiomers derived from anti-tumor antibiotics using chiral CE [27], but they could not apply this method in a quantificative fashion. Recently, GC/MS and CE/MS methods have been applied in a comparison study to investigate AA profiling in plant cell cultures [28]. In these experiments the authors hydrolyzed biological samples to get AAs content. Dunn et al. described the method and necessity of hydrolyzing tissue samples for AA analysis in rat fibrosarcomas [29]. Soga et al. reported the quantitative analysis of AAs in urine [19]. However, sample preparations from plants or from urine were easier than those from human or animal tissues, so specific procedures for connective tissue preparation may be needed. Therefore, this study was developed to analyze AAs quantitatively in the connective tissue of human. This method was validated and applied to the connective tissue of patients with POP. Age-matched controls were used, and the concentration changes of 17 AAs were observed.

2. Experimental

2.1. Materials and reagents

Proline, phenylalanine, methionine, asparagine, glutamic acid, glutamine, alanine, valine, leucine, histidine, glycine, aspartic acid, threonine, tryptophan, *trans*-4-hydroxy-proline, arginine, and isoleucine (Fig. 1) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA), and (5*R*)-5-hydroxylysine dihydrochloride monohydrate was purchased from FLUKA (Sigma–Aldrich, Steinhein, France). Formic acid, sodium bromo hydride ($NaBH_4$), sodium hydroxide (NaOH), TES buffer (*N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid), and methionine sulfone (internal standard) were, also, obtained from Sigma–Aldrich Chemicals Co. (St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were purchased from Burdick & Jackson (Muskegon, MI 49442,

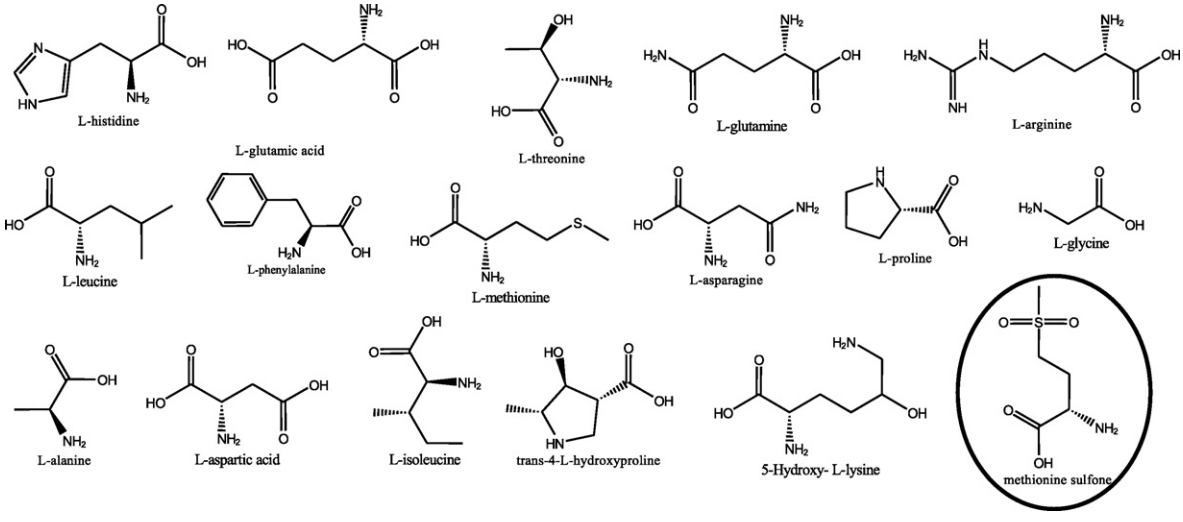

Fig. 1. Structures of 17 amino acids. (Round marked one is internal standard.)

Table 1
Mass spectrometry conditions for the determination of 17 amino acids and internal standard

Measured compounds	Parent ion (*m/z*)	Product ions (*m/z*)	Collision energy	Ionization mode
Proline	116	70	24	Positive
Phenylalanine	166	120	28	Positive
Methionine	150	133	26	Positive
Asparagines	133	87	24	Positive
Glutamic acid	148	130	26	Positive
Glutamine	147	130	26	Positive
Alanine	90	90	6	Positive
Valine	118	72	26	Positive
Leucine	132	86	24	Positive
Arginine	175	158	28	Positive
Histidine	156	110	28	Positive
Threonine	120	74	24	Positive
Glycine	76	76	6	Positive
Aspartic acid	134	116	24	Positive
Hydroxyproline	132	86	26	Positive
Isoleucine	132	86	24	Positive
Hydroxylysine	163	145	22	Positive
Methionine sulfone (ISTD)	181	136	26	Positive

USA). All chemicals were of analytical grade or HPLC grade and were used upon receipt. HPLC-grade water was purified using a Milli-Q purification system (Millipore; Bedford, MA, USA).

2.2. Instrumentation

2.2.1. CE and MS

A Beckman coulter MDQ CE system (Beckman instruments, Fullerton, CA, USA) connected to a Thermo LCQ Deca MS (Thermo, SanJose, CA, USA) with the ESI source was used to perform this study. Data analysis was performed with *X-caliber* (Thermo, SanJose, CA, USA), and statistics were calculated by Student's *t*-test.

2.2.2. CE and MS conditions

CE was performed using a 50 μm (i.d.) × 52 μm (o.d.) × 80 cm (length) untreated silica capillary (polymicrotechnology, Phoenix, AZ, USA). The temperature controlled capillary chamber was maintained at 20 °C by employing a liquid coolant. The capillary was conditioned with 0.1 M NaOH for 30 min and with the running electrolyte for 3 h consecutively before analysis. The stock solutions were prepared with 1 M formic acid to obtain a good background current. Before each injection, the silica capillary was preconditioned with the running electrolyte for 10 min. A Nanospace 3001 S1-2 (Shiseido Fine Chemicals, Tokyo, Japan) pump was used to deliver sheath liquid (50% methanol) at a rate of 10 μL/min.

Samples were injected for 1 min with a pressure of 0.8 psi. Separation was carried out with 1 M formic acid at a pressure of 1 psi, and 25 kV was applied during separation. Pressure together with voltage was applied to increase the separation efficiency of the method and decrease migration time of each amino acid so that the entire analysis time can be saved.

The MS was operated in positive ionization and selective reaction monitoring (SRM) modes in order to monitor specific parent-to-product transitions. Spray voltage and current was 5 kV and 80 μA, respectively. Detection was carried out at a sheath gas flow rate of 8 arb (arbitrary unit), where capillary ion spray voltage and ion capillary temperature were 5 kV and 275 °C, respectively. Prior to sample analysis, parent ion and product ion for each amino acid were tuned, and collision energy was optimized. Parent ion, product ion, and collision energy of the 17 AAs and internal standard are listed in Table 1.

2.3. Preparation of stock and working solutions

A stock solution for each analyte and mixture was prepared at a concentration of 20 μmol/mL (as the base in case of salt) in 1 M formic acid. To get the working solution (ranged from 0.1 to 10 μmol/mL), the stock solution was diluted serially with 1 M formic acid. A 10 μmol/mL stock solution of the internal standard (methionine sulfone) was also prepared in 1 M formic acid. 1 M formic acid, 0.1 N NaOH, 0.15 M TES buffer, 0.015 M TES buffer, and $NaBH_4$ were prepared with HPLC-grade water.

2.4. Sample preparation

A complex sampling procedure was followed to extract AAs from the pelvic connective tissue samples. A total of 100 mg of tissue was homogenized with 1 mL cold TES buffer (0.015 M) using HS-305 Homogenizer stirrer (Daihan Scientific, Seoul, South Korea), and the homogenate was centrifuged at 3000 × *g* for 15 min at 4 °C. After discarding the supernatant, the pellet was again centrifuged with 1 mL of cold TES buffer (0.015 M). Centrifugation was repeated three times. After centrifugation, the pellets were lyophilized for 24 h to dry them using a Vacuum Freeze Dryer (BioTron Co., Ltd. Puchon, South Korea). Ten microliters of ISTD was added to 3 mg of the dried connective tissue and suspended in 200 μL cold TES buffer (0.15 M) for 20 min at room temperature. Suspended samples were then reduced with 3 μL of $NaBH_4$ (10 mg/mL, 1% of the sample weight) for 40 min at room temperature. After reduction, the samples were hydrolyzed with 600 μL of freshly prepared 6 N

HCl (5 mg/mL of the sample weight) and kept in a heating block at 105 °C for 24 h. Hydrolysates were dried in a vacuum freeze dryer and dissolved in 200 μL of 1 M formic acid. Diluted samples were filtered through 0.2 μm nylon 66-syringe filter (Whatman, Kent, UK), and finally aliquots of 100 μL were transferred to vials for analysis.

2.5. Assay validation

2.5.1. Calibration and quality control samples

The calibration standards of the 17 AAs for tissue were constructed by spiking the appropriate amounts of standard AAs mixture in water, as it is not possible to get blank tissue without endogenous AAs, which could interfere, in the overall analysis. The calibration range was variable for each AA and was in the range from 0.05 to 10 μmol/mL. Calibration standards were prepared according to the procedure followed for tissue. The limit of detection (LOD) and limit of quantitation (LOQ) were defined as a signal to noise ratio of 3:1 and 10:1, respectively.

Quality control samples were made at three different concentrations (low, medium, and high concentrations) within the calibration range by spiking the appropriate amount of standard AAs mixture in water and were prepared by the same method as the tissue preparation. With the QC samples, accuracy and precision (reproducibility) of the assay was validated.

2.5.2. Precision and accuracy

Intra-day precision and accuracy were determined from the variability of three replicate analyses of quality control samples, analyzed within the same analytical run, and inter-day precision and accuracy were determined from the variability of replicate analysis quality control samples analyzed for 3 consecutive days.

2.5.3. Matrix effect and recovery test

The matrix effect test was designed following the standard addition method [30]. In a known concentration matrix, various amounts of standard were added. By comparing the differences between observed and expected concentrations the matrix effect was calculated.

The actual recoveries of the 17 AAs were obtained by spiking the analytes with concentrations that were the same as the quality controls.

The entire sample for the matrix effect and recovery test was prepared and analyzed using the same method as the tissue preparation.

2.5.4. Stability test

The stability during sample handling was verified by subjecting samples to three freeze–thaw cycles and storage for 7 days in the refrigerator at 4 °C prior to analyses.

Quality control samples at three concentrations (0.05, 1, and 10 μmol/mL) were utilized for this stability test.

2.6. Sample information

The study population consisted of 26 patients who visited the Urogynecology clinic of Yonsei Health System between September 2006 and December 2006. All subjects were divided into two groups: the POP patients group (defined as POP stage ≥II) and the control group (defined as POP stage 0). Each patient underwent a standardized urogynecological history interview and complete physical examination. All patients had pelvic examinations performed both in the supine position and in a 45° upright position in a birthing chair while performing the Valsalva maneuver with maximum effort. Pelvic organ prolapse was quantified according to the International Continence Society's Pelvic Organ Prolapse Quantification (POP-Q) system [31]. Age, parity, gravity, and body mass index (BMI) were investigated in all subjects. All patients were of menopausal status, and no patient had received hormonal replacement therapy before. This study was approved by the Institutional Review Board at Yonsei Health System.

After informed consent was obtained, approximately 1 cm^3 full-thickness uterosacral ligaments were excised from the area connected to the uterus during the operation immediately after hysterectomy from patients and control women. The obtained uterosacral ligaments were kept at −80 °C. The general characteristics of the patients and controls included in this study are listed in Table 2.

Table 2
General characteristics of patient and control groups

Characteristics	Tissue	
	POP	Control
No.	21	13
Age	67.5 ± 8.0	62.2 ± 5.5
Parity	4.4 ± 2.5	3.1 ± 1.4
Gravity	6.2 ± 2.7	4.8 ± 1.5
Menopause (%)	100%	100%

3. Results and discussion

3.1. CE-ESI–MS/MS analysis

All the AAs were clearly separated within 20 min as shown in Fig. 2. Parent ions and product ions were used to identify and quantify each AA.

3.2. Stability test

The stock solutions of 17 AAs, standards, and the internal standard (methionine sulfone) were stable over three freeze–thaw cycles and refrigerated storage for 7 days at 4 °C prior to analysis. All results were in the acceptable range of ±15% deviation from the nominal concentrations.

3.3. Matrix effect and recovery test

The results of matrix effect and recovery are presented in Table 3a and b. The matrix effects for the compounds were within ±15.4%. Recoveries of all AAs were greater than 84.3% at all concentration ranges.

Table 3
Matrix effect, recovery, intra-day and inter-day validations for amino acids in tissue

Compounds	Concentrations (μmol/mL)	Matrix effect (%)	Recovery (%)	Intra-day ($n=3$)		Inter-day ($n=3$)	
				CV (%)	Accuracy	CV (%)	Accuracy
Proline	0.1	12.9	91.7	9.9	116.7	4.4	114.7
	1	12.2	93.7	9.2	89.0	9.1	88.7
	3	3.8	100.7	7.1	95.4	8.3	95.2
Phenylalanine	0.1	3.4	108.9	5.4	113.0	7.2	91.0
	1	12.8	109.5	12.0	88.0	10.6	94.3
	3	8.7	99.7	5.2	95.8	2.1	100.0
Methionine	0.5	15.4	98.3	2.9	118.0	4.6	116.1
	1	7.7	115.7	4.9	116.7	14.4	113.6
	3	−9.1	102.9	3.1	112.2	3.7	109.6
Asparagine	0.5	2.1	97.9	8.5	88.7	6.9	87.6
	1	−2.1	99.1	6.7	102.0	2.1	97.0
	3	−6.9	104.1	4.4	102.6	4.7	98.6
Glutamic acid	0.1	0.0	92.1	9.1	110.0	17.8	116.6
	1	2.6	104.4	5.9	85.7	7.5	88.3
	3	7.7	90.1	0.2	93.6	7.3	95.6
Glutamine	2	2.9	103.7	2.2	110.3	2.3	120.6
	4	8.9	100.3	4.5	91.5	7.1	88.7
	8.5	5.5	101.5	6.5	95.1	3.4	95.9
Alanine	0.5	12.6	84.3	3.7	88.3	6.2	86.0
	4	1.3	103.7	2.3	100.5	5.1	90.3
	10	−2.9	102.3	7.4	94.6	4.9	95.8
Valine	0.5	9.2	88.4	8.2	84.1	13.2	88.7
	4	15.1	102.1	6.8	94.9	2.9	93.2
	10	2.7	103.7	4.9	99.4	10.6	96.6
Leucine	0.05	13.2	114.3	12.1	108.2	13.3	98.4
	0.5	11.5	97.4	12.6	87.3	2.9	104.7
	3	14.1	103.2	6.5	103.3	5.9	103.8
Arginine	0.1	11.3	89.2	9.1	110.0	16.7	120.0
	1	8.8	98.9	4.6	97.7	12.6	107.7
	3	3.5	87.6	1.2	102.0	3.9	104.3
Histidine	0.5	5.5	106.7	7.1	112.0	9.1	110.0
	1	2.0	109.2	1.6	109.0	5.6	104.7
	3	1.2	97.7	3.4	96.7	6.4	96.4
Threonine	0.1	5.4	93.5	8.6	99.1	14.2	105.7
	1	6.1	104.5	10.2	90.0	14.3	103.0
	3	14.7	100.9	2.3	85.1	14.3	87.4
Glycine	0.5	−6.3	80.9	10.1	86.0	15.9	85.8
	4	3.3	99.7	6.4	98.3	1.0	94.3
	9.5	5.2	100.4	0.6	99.9	1.1	99.2
Aspartic acid	0.1	9.1	92.5	14.4	120.0	13.1	116.7
	1	−3.2	96.9	6.4	86.7	5.1	85.0
	3	14.6	86.8	12.8	90.6	13.7	86.7
Hydroxyproline	0.05	−7.3	121.9	16.8	116.0	19.0	112.5
	1	−1.1	88.3	8.6	88.3	7.2	103.7
	3	13.1	102.9	6.4	108.9	6.3	107.9
Isoleucine	0.5	2.8	120.0	19.5	106.7	13.1	80.3
	1	3.8	98.4	5.5	87.5	8.1	111.3
	3	3.7	103.9	3.6	103.1	3.1	106.7
Hydroxylysine	0.5	14.0	82.6	1.3	80.7	7.2	89.3
	1	15.0	104.1	4.1	100.7	8.2	100.6
	3	0.0	99.0	2.8	100.4	2.4	101.6

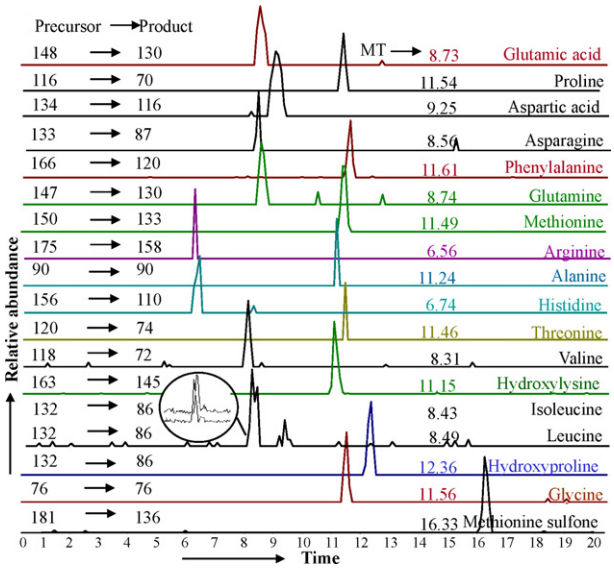

Fig. 2. Extracted ion electropherograms of 17 amino acids and methionine sulfone (internal standard) at 1 μmol/mL.

3.4. Method validation

3.4.1. Linearity, calibration range and LLOQ

The calibration curves for the 17 analytes were analyzed by weighted ($1/x^2$) least-squares linear regression. Regression co-efficients (R) of the calibration curves were greater than 0.993 for all AAs in the range of 0.05–10 μmol/mL (Table 4). The lower limit of quantitation (LLOQ) was variable and in the range of 0.05–2 μmol/mL.

3.4.2. Precision and accuracy

The intra-day ($n = 3$) and inter-day ($n = 3$) variabilities for all analytes are also summarized in Table 3a and b. The acceptable quantification range varied in the range of 0.05–10 μmol/mL for the 17 AAs. In the quantification, QC samples showed good precision and accuracy within the deviation range of ±20% in low concentration. For the middle and higher concentrations, the precision and accuracy were within ±15% and 85–115%, respectively.

Table 4
Calibration range, regression equation and regression co-efficient (R) of 17 analyzed amino acids

Compounds	Range (μmol/mL)	Regression equation	R
Proline	0.1–3	$y = 2.2924x - 0.1532$	0.998
Phenylalanine	0.1–3	$y = 19.699x - 0.5498$	0.999
Methionine	0.5–3	$y = 4.2481x - 2.46$	0.997
Asparagine	0.5–3	$y = 1.1018x - 0.0004$	0.999
Glutamic acid	0.1–3	$y = 5.9843x - 0.7465$	0.993
Glutamine	2–10	$y = 2.3455x - 3.8328$	0.997
Alanine	0.5–10	$y = 1.6528x - 0.0378$	0.999
Valine	0.5–10	$y = 2.9633x + 0.2136$	0.999
Leucine	0.05–3	$y = 15.52x + 0.12398$	0.999
Arginine	0.1–3	$y = 3.4175x - 0.1327$	0.998
Histidine	0.5–3	$y = 8.0905x - 3.406$	0.994
Threonine	0.1–3	$y = 0.8877x - 0.0454$	0.999
Glycine	0.5–9.5	$y = 0.8168x + 0.1676$	0.999
Aspartic acid	0.1–3	$y = 3.9727x - 0.3846$	0.995
Hydroxyproline	0.05–3	$y = 13.563x + 0.1951$	0.999
Isoleucine	0.1–3	$y = 14.652x + 0.9192$	0.998
Hydroxylysine	0.5–3	$y = 4.3829x - 0.6642$	0.997

Table 5
Comparison of calculated tissue amino acids among POP and control

Compounds	Tissue (mean ± S.D.)		p-Value
	POP	Control	
Proline	3005.6 ± 1098.2	3076.6 ± 1958.4	ns
Phenylalaine	712.5 ± 210.7	595.0 ± 290.8	ns
Methionine	1408.5 ± 95.7	1150.5 ± 361.8	<0.03
Asparagine	761.5 ± 508.8	836.5 ± 231.9	ns
Glutamic acid	6141.6 ± 315.0	1104.6 ± 655.8	ns
Glutamine	4510.1 ± 425.4	3514.2 ± 1231.2	<0.01
Alanine	2833.9 ± 878.5	3083.0 ± 2361.5	ns
Valine	1535.8 ± 565.5	1545.0 ± 842.4	ns
Leucine	1065.4 ± 377.8	1066.7 ± 625.4	ns
Arginine	1915.5 ± 513.1	2000.5 ± 1229.2	ns
Histidine	1182.5 ± 147.6	938.2 ± 313.4	<0.02
Threonine	865.1 ± 302.5	824.1 ± 444.9	ns
Glycine	7236.6 ± 2858.4	8048.4 ± 7296.4	ns
Aspartic acid	919.3 ± 235.3	889.1 ± 511.8	ns
Hydroxyproline	1203.5 ± 413.7	1123.9 ± 768.6	ns
Isoleucine	1167.6 ± 441.2	1109.9 ± 637.1	ns
Hydroxylysine	368.3 ± 152.9	381.9 ± 104.7	ns

*"ns" = "not significant".
**"nd" = "not detected".
Concentrations are present in μmol/kg of tissue.

3.5. Application on POP

Female pelvic floor dysfunction, such as POP, is a major health and quality-of-life problem for aging women in their menopausal years. Weakening of pelvic supportive tissues is thought to be a contributing etiology in POP [22,23,32]. The pelvic muscles of the pelvic floor and their intact attachment to the pelvic fascia support pelvic organs [33]. Many investigators have suggested that a decrease level of collagen and altered morphological features could be the main contributing factor for POP [24–26,34].

Abnormalities in collagen metabolism have also been identified in association with another kind of pelvic floor disorder, stress urinary incontinence [35].

Major component AAs for collagen are glycine, proline, hydroxyproline, and hydroxylysine [36]. Therefore, we first tried to find the relations between POP and those AAs and then the effect of other AAs on POP. Most of the quantification for collagen has been carried out using confocal microscopy [37] or immunohistochemistry [38], but the correct quantitative determination of AAs within collagen has not been evaluated. This is the first quantitative evaluation of AAs in patients with POP. The 17 AAs were quantified well in pelvic tissue, and the concentrations were compared between patients and age-matched controls. In tissues the mean concentration of proline, hydroxylysine, and glycine decreased in patients compared to the controls; however, these differences were not significant. These results do not prove a direct relationship between collagen and POP. On the other hand, the concentrations of glu-

tamine ($p < 0.01$), methionine ($p < 0.03$), and histidine ($p < 0.02$) were significantly higher in the pelvic tissues of POP patients. Isoleucine, threonine, phenylalanine, aspartic acid, and glutamic acid levels were higher in POP patients, and asparagine, alanine, valine, and arginine levels were lower in the tissue of POP patients; however, these differences were also not significant. These results are presented in Table 5.

Notably, the specific amino acid for collagen, hydroxyproline, has been used to quantify collagen concentration and to observe the extent of POP [39]. From this, we expected that it was possible to observe a significant decrease of hydroxyproline concentration and other major component AAs of collagen in the pelvic tissue of POP patients. There were no significant differences in the concentrations of major component AAs of collagen between the two groups despite of our expectation. However, a number of unexpected AAs were significantly increased. From the several reports we found, we have determined that the relation between collagen and POP is not straightforward. Gabriel et al. did not find a difference in collagen I expression between patients and controls, whereas the expression of collagen II was significantly related to the presence of POP [38]. Drutz et al. studied a different factor, the metabolism of elastin, which is one of the components of pelvic connective tissue, and found that it may contribute to the alterations and dysfunction of pelvic connective tissue [40]. These reports urge that other factors in pelvic tissue could affect the pathogenesis of POP.

Thus, it is hard to suggest that collagen might be a major factor in the genesis and development of POP. Other factors may affect POP and should be investigated to reveal the mechanism of POP pathogenesis. Despite not showing a clear relation between POP and AAs in this study, we successfully measured 17 AAs in tissue with some meaningful results, though the results were not sufficient to elucidate the cause of POP. Further studies with larger control and patient groups should be conducted to reveal the exact role played by AAs in the pathogenesis of POP.

4. Conclusion

A reliable and reproducible quantitative analytical method for 17 AAs in tissue has been described in POP patients. Seventeen AAs within connective tissue were simultaneously determined and quantified. To our knowledge, this is the first example of connective tissue being analyzed using CE-ESI–MS/MS. The method demonstrated good reliability and reproducibility. Average recoveries of the 17 AAs were good, and the matrix effect was minimal. This method was successfully applied to quantify AAs in pelvic tissue of POP patients. These results indicate that this method, which utilizes CE-ESI–MS/MS, can be applied on tissue samples to analyze AAs.

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