

This article was downloaded by:

On: 23 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

Proteomics of Collagen Peptides: A Method to Reveal Minor Changes in Post-Translationally Modified Collagen by HPLC and Capillary Electrophoresis

Adam Eckhardt^a; Ivan Mikšík^a; Jana Charvátová^a; Zdeněk Deyl^a; Esther Forgács^b; Tibor Cserhádi^b

^a Institute of Physiology, Academy of Sciences of the Czech Republic, Praha, Czech Republic ^b Institute of Chemistry, Chemical Research Centre, Hungarian Academy of Sciences, Budapest, Hungary

To cite this Article Eckhardt, Adam , Mikšík, Ivan , Charvátová, Jana , Deyl, Zdeněk , Forgács, Esther and Cserhádi, Tibor(2005) 'Proteomics of Collagen Peptides: A Method to Reveal Minor Changes in Post-Translationally Modified Collagen by HPLC and Capillary Electrophoresis', *Journal of Liquid Chromatography & Related Technologies*, 28: 10, 1437 – 1451

To link to this Article: DOI: 10.1081/JLC-200058322

URL: <http://dx.doi.org/10.1081/JLC-200058322>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Proteomics of Collagen Peptides: A Method to Reveal Minor Changes in Post-Translationally Modified Collagen by HPLC and Capillary Electrophoresis

Adam Eckhardt, Ivan Mikšík, Jana Charvátová, and Zdeněk Deyl
Institute of Physiology, Academy of Sciences of the Czech Republic,
Praha, Czech Republic

Esther Forgács and Tibor Cserhádi
Institute of Chemistry, Chemical Research Centre, Hungarian Academy
of Sciences, Budapest, Hungary

Abstract: Depository effects in slowly metabolized proteins, typically glycation or the estimation of products arising from the reaction of unsaturated long chain fatty acid metabolites (possessing aldehydic groups) are very difficult to assess owing to their extremely low concentration in the protein matrix. Posttranslational nonenzymatic modifications of collagen with sugars and oxidation products of lipid metabolism in tail tendons of two groups of rats (controls and hypertriglyceridemics with a high fructose diet) were studied. Collagen (a mixture of type I and III) was digested by bacterial collagenase and separated by reversed-phase HPLC; the profile obtained was divided into five fractions, which were further characterized by capillary electrophoresis (CE) in a bare fused silica capillary (37/30 cm × 75 μm I.D.). As the chromatographic and electromigration behaviour of individual peptides was considerably different, the combination of HPLC and CE appeared as a suitable approach capable of acceptable separation of complex peptide mixtures (over 150 peptide peaks were obtained on the electropherograms). This two-stepped peptide mapping with subsequent statistical evaluation (linear regression analysis) was shown to represent a

Address correspondence to Ivan Mikšík, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Praha 4, Praha, Czech Republic. E-mail: miksik@biomed.cas.cz

reliable approach for revealing posttranslational modifications in slowly metabolized model proteins *in vivo*.

Keywords: Proteomics, Collagen peptides, HPLC, Capillary electrophoresis

INTRODUCTION

Linear and multiple linear regression analyses have been extensively used to find the relationship between one dependent and one or more independent variables. Because of their simplicity and good predictive power they have been successfully applied to various chromatographic techniques.^[1] These methods have been recently employed in chromatography for the investigation of the molecular mechanism of separation,^[2] for the classification of modern stationary phases,^[3] for quantitative structure-retention relationship studies in HPLC^[4] and in GC,^[5,6] and for the correlation of the retention behavior and biological activity.^[7]

In protein chemistry peptide mapping, both by chromatographic and electromigration methods, is a widely applied approach.^[8] Unfortunately, current separation methods do not exhibit sufficient selectivity to offer baseline separations in complex peptide mixtures. There are a number of situations, in which proteins present in tissues undergo minor non-enzymatic modifications, the assessment of which is extremely difficult.

Owing to its slow metabolic turnover, collagen is typical in its capability to accumulate reactive metabolites through enzymatically nonregulated reactions, and its peptide maps can serve as model mixtures for the principle component analysis (PCA) studies aimed at revealing the presence or absence of such minor (though biologically quite important) modifications. Glycation and formation of advanced glycation end products (AGEs) represent typical examples.^[9] In spite of numerous efforts, the chemical nature of the arising products (except pentosidine) remains unknown.^[10]

The main problem of assaying these minor modifications is based on the fact that the arising compounds (adducts) are unstable under conditions under which collagens can be chemically hydrolyzed to constituting amino acids. Luminiscence based methods, though they added to the basic information about the arising products, are not selective enough even if the synchronous luminiscence approach is applied.^[10]

Cyanogen bromide cleavage, which is routinely used to bring connective tissue collagen into solution could also not be exploited, as our preliminary experiments revealed that, in general, the selectivity of these techniques, *i.e.*, flat bed gel and capillary electrophoresis (CE), as well as reversed phase HPLC (RP-HPLC), if used as the single separation steps are not capable to reveal the few modifications occurring in the molecule.^[11] This is particularly true with the high molecular mass fragments, the modification of which does not bring about sufficient alteration of the physico-chemical properties

that would offer distinct changes in the chromatographic or electrophoretic profile.

For this reason, we applied deep cleavage of the parent collagen molecules with *Clostridium histolyticum* collagenase. This protease produces a very complex peptide mixture (theoretically about 172 peptides could arise from the naturally occurring collagen type I and III mixture). This set of peptides can be partially separated by CE in a bare-silica capillary at acid pH with a selectivity sufficient to indicate differences in individual peptide profiles, however, it has to be kept in mind that many of the peaks seen in the well reproducible electrophoretic profiles need not represent pure peptides (only 65 peptide peaks were discernible in CZE).^[12]

The objectives of our study were (i) the separation of peptides in various collagen hydrolysates using a combination of RP-HPLC and CZE, (ii) the quantitative evaluation of the relative concentration of peptides using two different integration methods, and (iii) revealing the similarities and dissimilarities of the peptide profiles in the electropherograms by PCA linear regression analysis. Bacterial collagenase digests of crude tail tendon collagen samples composed of collagen type I and III, obtained from controls and genetically hypertriglyceridemic animals kept simultaneously for a period of time on a high fructose diet (experimental group), served as models for extensive post-translational modifications. It was expected that in the experimental group some of the available amino groups of the protein molecule will be modified by the reaction with the free oxo-moiety possessing compounds, i.e.; fructose and/or lipid metabolites.

EXPERIMENTAL

Chemicals

All chemicals used were either of analytical grade or highest available purity. Calcium chloride was obtained from Lachema (Brno, Czech Republic), collagenase E.C. 3.4.24.3., 0.8 U/mg, from *Clostridium histolyticum* (c.n. 29676), was from Fluka (Buchs, Switzerland), phosphate buffer 100 mmol/L, pH = 2.5, with polymer modifier was purchased from Bio-Rad Laboratories (Hercules, CA, USA; c.n. 148–5010), acetonitrile (HPLC gradient grade) was purchased from Merck (Darmstadt, Germany). The peptides used for spiking identification (KGP—lysine-proline-glycine; GPR—glycine-proline-arginine; NPG—asparagine-proline-glycine; GPQ—glycine-proline-glutamine) were obtained from Polypeptide Laboratories (Prague, Czech Republic). All other chemicals (inclusive of gemfibrozil) and the GPA (glycine-proline-alanine) peptide were obtained from Sigma (St. Louis, MO, USA). All solutions were prepared in Mili-Q Water (Millipore, Bedford, MA, USA).

Tissues and Animals Used

Tendons were dissected from tails of six month old female Wistar strain rats (six per group).

Two groups of rats were compared: 1. Controls (group C). 2. Hereditary hypertriglyceridemic (HTG) rats kept on high fructose diets; the last three weeks before being used for the experiment the rats were kept on water containing 10% fructose (group HTG + F).

To obtain peptide maps collagen was digested by bacterial collagenase (collagen/collagenase ratio, 100:1, w/w). The samples were incubated at 37°C for 48 h in the collagenase activating buffer (200 mmol/L NH_4HCO_3 , 1 mmol/L CaCl_2 , pH = 7.8).

Apparatus and Operating Conditions

Reversed-Phase HPLC

An HPLC system HP 1100 (from Hewlett Packard, Fullerton, CA, USA) was used. Chromatographic separation was carried out on the Zorbax Eclipse XDB C18 column (150 × 2.1 mm, particle size 5 μm) (Hewlett Packard).

Separation was achieved by a linear gradient between mobile phase A (water-trifluoroacetic acid, 100:0.1, v/v) and B (acetonitrile-trifluoroacetic acid, 100:0.08, v/v). Linear gradient from 0% B to 50% B at 28 min was applied, followed by 10 min elution with 100% B. Flow-rate was 0.25 mL/min, column temperature was held at 25°C, and UV detection was done at 214 nm.

Five fractions were collected (the first: from 0 to 10 min of retention time, the second: from 10 to 12 min, the third: from 12 to 17 min, the fourth: from 17 to 20 min, and the fifth: from 20 to 38 min), see Fig. 1. The fractions were lyophilized and reconstituted in water to the total volume of 50 μL . It has to be emphasised that care must be taken about lyophilization, which can be used as a sample concentration step only. Taking the individual fractions to complete dryness brings about problems with redissolving this material (typically in the second HPLC fraction).

Capillary Electrophoresis

A Beckman P/ACE 5000 capillary electrophoresis (Fullerton, CA, USA) was used throughout this study.

Separations were run at 10 kV in a bare fused silica capillary (37 cm, 75 μm I.D., 30 cm to the detector) at 20°C. UV absorbance at 200 nm was used for detection.

Phosphate buffer 100 mmol/L, pH = 2.5, with a polymer modifier (Bio-Rad Laboratories, Hercules, CA, c.n. 148–5010) was used as background electrolyte.

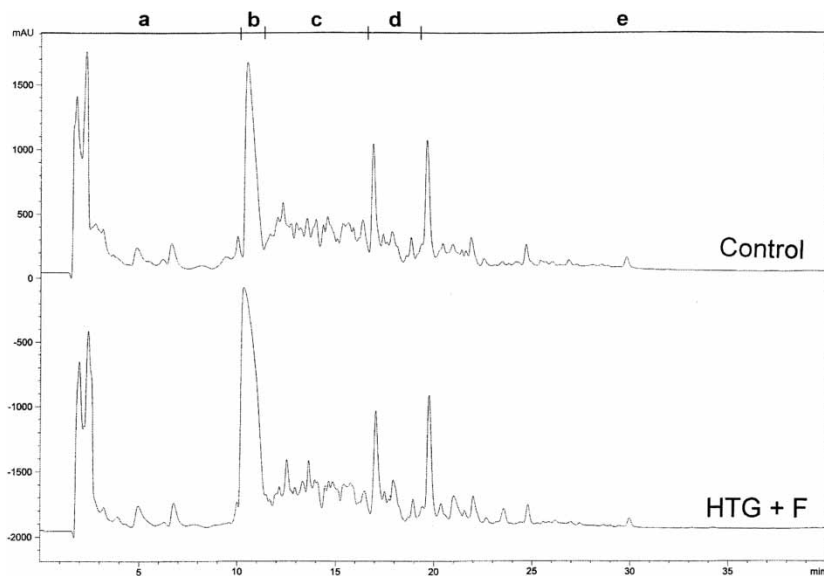


Figure 1. HPLC profiles of the bacterial collagenase digest of collagen samples (naturally occurring mixture of collagen type I and III of rat tail tendons) of controls (A) and HTG + F treated animals (B). Letters refer to fractions collected for further separation by capillary electrophoresis and fraction sizes are identical for controls and HTG + F samples. For details see Experimental section.

The samples were diluted with the run buffer (sample/buffer, 1:2, v/v). Injection was done hydrodynamically by overpressure (3.45 kPa, 1 s). Before analysis the capillary was conditioned by the run buffer (1 min). After every run the capillary was flushed step-wise with the run buffer (1 min), water (1 min), 1 M NaOH (3 min), water (1 min), 3 M HCl (1 min) and water (1 min).

Separation and Analysis of Peptides

The preliminary separation of peptides of control and treated samples had been carried out by HPLC. The chromatograms were cut in five fractions (see Fig. 1), and the peptides in each fraction were further analysed by CE. In order to compare the peptide profiles of samples, the electropherograms have been divided into five sections (different from those obtained by HPLC). Sub-sectioning of the electrophoretic profiles (five sections per each electropherogram) was different in each chromatographic fraction because of the peak distribution (this is clearly indicated in the respective Figs. 2 and 3). The peptide content of the individual electrophoretic sections was measured by baseline and valley-to-valley integrations, and the relative

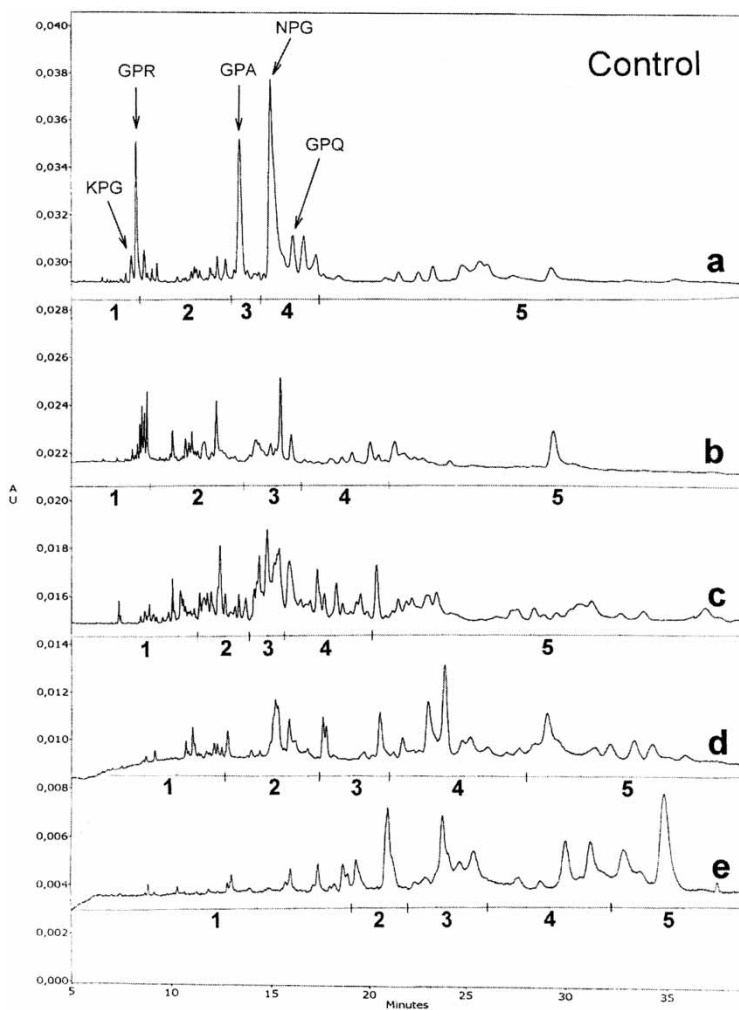


Figure 2. Capillary electrophoretic profiles of individual fractions accumulated from the HPLC runs of control samples (*a–e*). Numbers at individual recordings (1–5) refer to fractions indicated in Fig. 1. Further sub-sectioning of the electrophoretic profiles is clearly indicated in the Figures. Five, typically collagenous peptides, were identified in the first chromatographic section (*a*) as indicated.

amount (in percentage) of peptides in the sections was calculated taking the whole area of the electropherogram as 100%. Each measurement was carried out in five parallel determinations and the mean values were calculated. In order to compare the capacity of the two methods of integration for the differentiation between the peptide profiles of control and treated

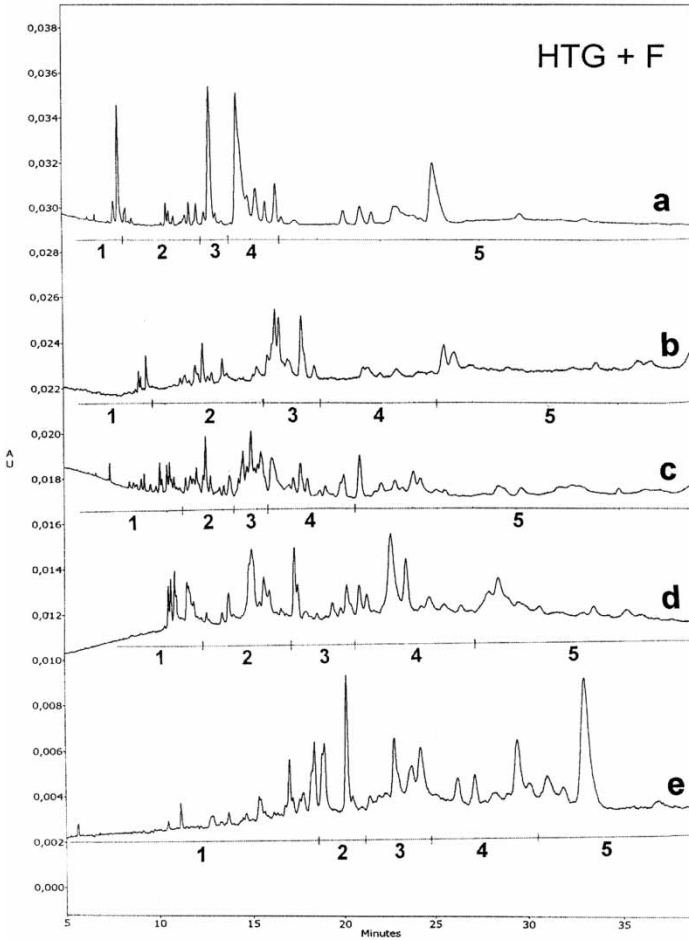


Figure 3. Capillary electrophoretic profiles of individual fractions accumulated from the HPLC runs of HTG + F samples. Numbers at individual recordings refer to fractions indicated in Fig. 1. Further sub-sectioning of the electrophoretic profiles is indicated in the Figures and is the same as used for controls (compare Fig. 2).

samples, linear regression analysis was applied three times: (i) peak areas calculated by the baseline integration method only (equation 1), (ii) peak areas calculated by the valley to valley integration method only (equation 2), and (iii) peak areas calculated by the both methods (equation 3) being included into the regression analysis. Outliers (peptide sections highly differing significantly in control and treated samples) were detected by calculating the confidence limit of 95% significance level for each linear regression.

RESULTS AND DISCUSSION

Profiling of bacterial collagenase digests of both control and HTG + F rat tail tendons by HPLC revealed very complex profiles (Fig. 1) containing five dominant peaks. Because both types of profiles were hardly distinguishable (if at all), five fractions (*a–e*) were collected from each category of samples (controls and HTG + F), for further separation by capillary electrophoresis under conditions specified in Experimental section. Collection times of individual fractions are schematically drawn in the Figure. The first fraction (*a*) contained the fast moving double peak; all the other fractions, except fraction *c*, contained one dominant peak. Fraction *c* contained a set of small peaks only; on the contrary, fraction *b* contained the respective dominant peak and was devoid of any of the small peaks. When we compare both groups of animals, we can observe that at least all the dominant peaks were eluted practically with the same retention time; minor, though well reproducible variations were observed between controls and HTG + F collagenase digests in the category of small peaks. Collecting of HPLC fractions followed identical time schedules, both for controls and the HTG + F samples.

After being subjected to capillary electrophoretic separation using pH 2.5 phosphate (100 mmol/L) buffer as background electrolyte, the profiles shown in Fig. 2 (plates *a–e*) for control, and Fig. 3 (plates *a–e*) for HTG + F samples were obtained.

Capillary electrophoresis was (if used without chromatographic pre-fractionation) capable of partial separation of about 65 peptide peaks from the original mixtures of both categories of collagen samples (controls and HTG + F) digested by bacterial collagenase. When the same original samples were separated by RP-HPLC, we obtained only about 45 peptide peaks (see Fig. 1).

By combining RP-HPLC and CE, over 150 peptide peaks on the electrophoregram were obtained; see Figure 2 and 3 (as compared to the theoretical 172 peptides in controls). It is, perhaps, unnecessary to stress that it is unlikely that most of the peaks seen both in the control and HTG + F digest were pure chemical entities.

The electrophoretic profiles of individual HPLC fractions were, for the purpose of calculation, further subdivided into five sections each (1–5). Because the distribution of electrophoretic peaks was considerably different in individual HPLC fractions, further sub-sectioning of these profiles was different for each profile obtained as indicated in Figs. 2 and 3, though it was the same for the control and HTG + F samples.

In order to be able to compare the control and HTG + F profiles, the relative peak areas of the clusters of peptides in the individual CE sections of treated and control samples were calculated (see Tables 1 and 2). These data indicate that the differences between the relative peak areas of control and treated samples in the majority of sections are low, suggesting the basic

Table 1. Average relative peak areas (in percentage) of peptides in the CE sections calculated by the baseline integration method

HPLC fraction no.	Group	CE section no. ^a				
		1	2	3	4	5
<i>a</i>	Control	5.09	8.79	11.81	43.41	31.00
	HTG + F	6.09	9.12	11.79	33.13	39.27
<i>b</i>	Control	8.59	21.52	18.69	14.15	37.05
	HTG + F	5.36	26.20	23.04	11.42	33.98
<i>c</i>	Control	7.34	11.25	17.62	22.30	41.48
	HTG + F	8.67	12.88	21.22	21.51	35.72
<i>d</i>	Control	6.11	17.15	12.61	35.35	28.79
	HTG + F	8.17	20.29	11.64	37.14	22.76
<i>e</i>	Control	11.44	12.75	25.67	26.81	23.33
	HTG + F	17.50	11.16	22.33	25.93	23.09

^aThe fact that the size of electrophoretic sections is different with individual HPLC fractions should be stressed (see Fig. 2).

similarities of the peptide profile of samples. The parameters of linear regressions are compiled in Table 3. The data in Table 3 entirely support our previous qualitative conclusions. Highly significant linear relationships were found between the peptide profiles of treated and control samples in each instance, proving again the basic similarity of the peptide profiles. This indicates clearly that there are only a few strongly preferred targets in the non-enzymatic modification in the HTG + F rats. On the other hand, it cannot be excluded that other targets, which are not discernible by the

Table 2. Average relative peak areas (in percentage) of peptides in the CE sections calculated by the valley-to-valley integration method

HPLC fraction no.	Group	CE section no.				
		1	2	3	4	5
<i>a</i>	Control	6.44	7.75	13.61	42.42	29.77
	HTG + F	8.75	8.35	16.13	39.06	27.11
<i>b</i>	Control	9.21	19.48	20.76	13.36	37.19
	HTG + F	6.78	25.12	23.78	7.85	36.58
<i>c</i>	Control	8.48	11.52	14.02	22.21	43.76
	HTG + F	10.06	13.23	16.85	17.85	41.92
<i>d</i>	Control	6.56	18.15	11.56	34.23	29.50
	HTG + F	8.48	23.40	10.82	33.06	24.23
<i>e</i>	Control	13.05	16.12	19.43	22.87	28.52
	HTG + F	21.65	13.84	15.28	21.67	27.55

Table 3. Parameters of linear regression between the relative peak areas of sections of control and treated samples. For number of equation see Experimental.

Parameter	Equation no.		
	1	2	3
n	25	25	50
a	-0.48	-0.16	-0.47
b	1.02	1.01	1.02
$r_{\text{calc.}}$	0.9317	0.9442	0.9393
$r_{99.9\%}$	0.6524	0.6524	0.4433

Eq. 1 is based on baseline integration method.

Eq. 2 is based on valley-to-valley integration method.

Eq. 3 is based on both the baseline and valley-to-valley integration methods.

$$\text{Relative peak area}_{\text{control}} = a + b \cdot \text{Relative peak area}_{\text{HTG+F}}$$

methods used, do not exist. The similarity of slope and intercept values demonstrate the similarity of integration methods as well. The plots of peptide profiles with the confidence limit of 95% significance level are shown in Figs. 4–6. The plots prove again the basic similarity of the peptide profile, however, outliers (indicators of significant differences, which are specific for baseline and valley-to-valley integration) can be

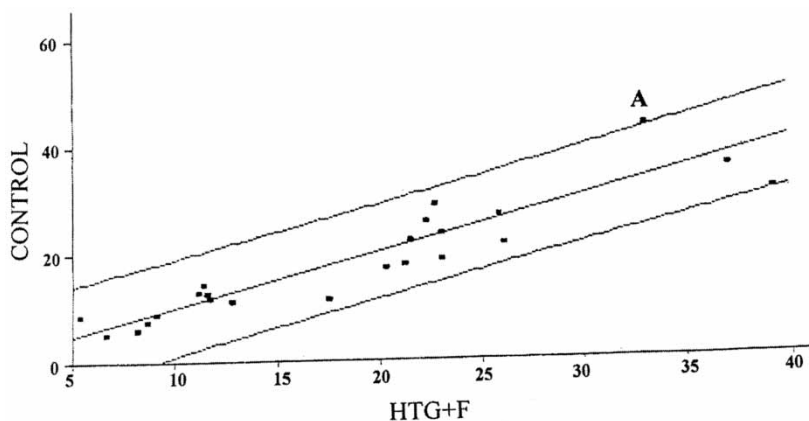


Figure 4. Relationship between the relative peak areas of peptides in control and HTG + F samples calculated by the baseline integration method. Upper and lower lines are the confidence limit of 95% significance level. Outlier A: fourth CE section of the first HPLC fraction.

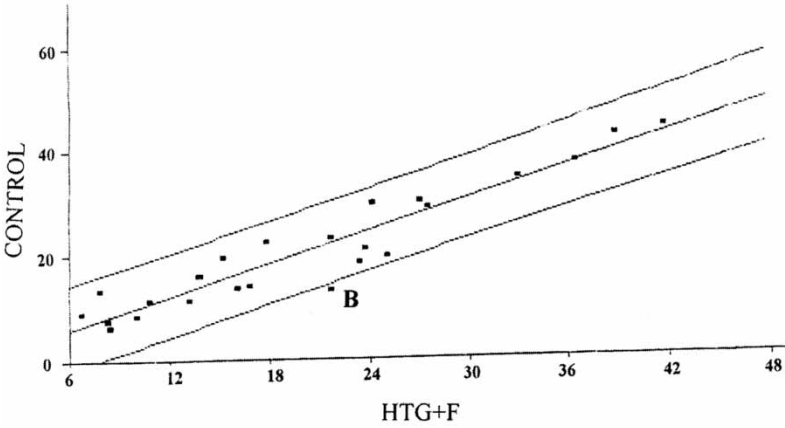


Figure 5. Relationship between the relative peak areas of peptides in control and HTG + F samples calculated by the valley-to-valley integration method. Upper and lower lines are the confidence limit of 95% significance level. Outlier B: first CE section of the fifth HPLC fraction.

found. Using the integration methods separately, only one outlier can be detected, while the simultaneous application of integration methods results in three outliers. This finding indicates that the reliability of differentiation between control and treated samples can be considerably enhanced by the simultaneous use of baseline and valley to valley integration methods and,

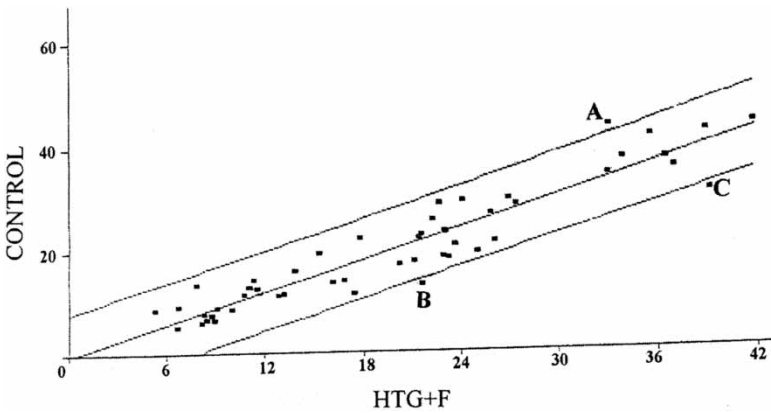


Figure 6. Relationship between the relative peak areas of peptides in control and HTG + F samples calculated by both (the baseline and the valley to valley) integration method. Upper and lower lines are the confidence limit of 95% significance level. Outliers A and B are the same as in Figures 5 and 6. Outlier C: fifth CE section of the first HPLC fraction.

Downloaded At: 18:52 23 January 2011

consequently, their simultaneous application in similar studies can be highly recommended.

Differences between the individual electrophoretic sections were shown to occur in sections 4 and 5 of the first chromatographic fraction (*a*) and in the first section of the chromatographic fraction *e*. Enlarged details of the relevant profiles are shown in Figs. 7–9. The first two differences were

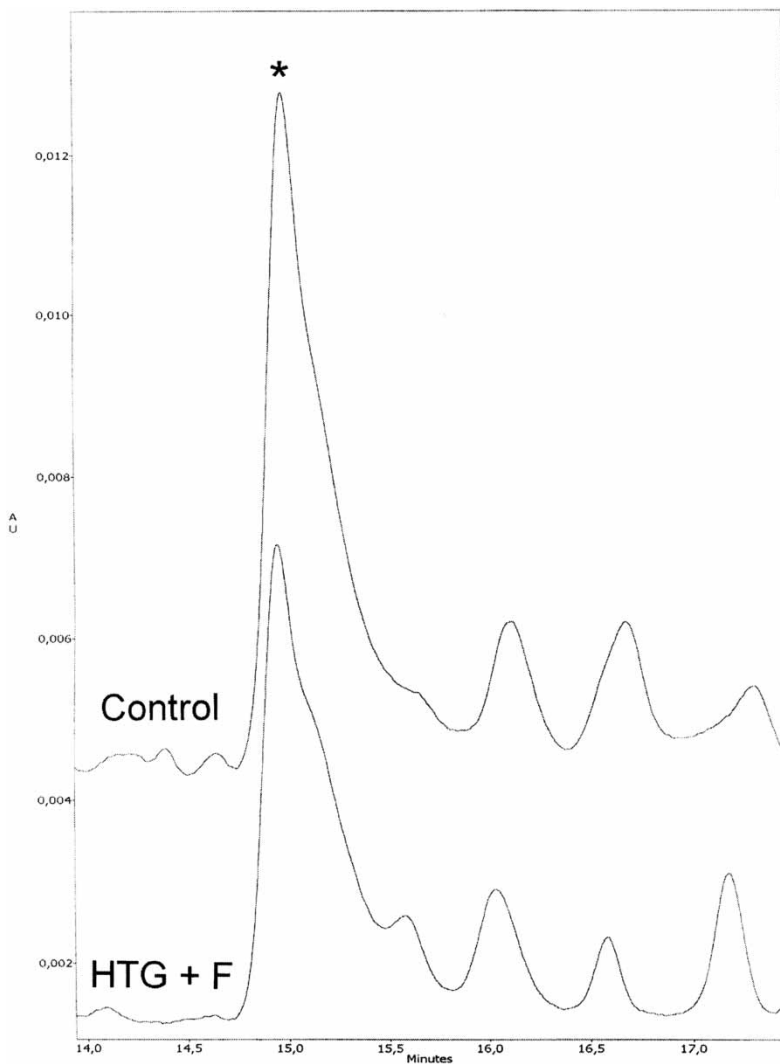


Figure 7. Enlarged sections of the fourth electrophoretic section (4) of the first HPLC fraction (*a*). An asterisk indicates the main difference. Note that the first peak is considerably lower in the HTG + F profile.

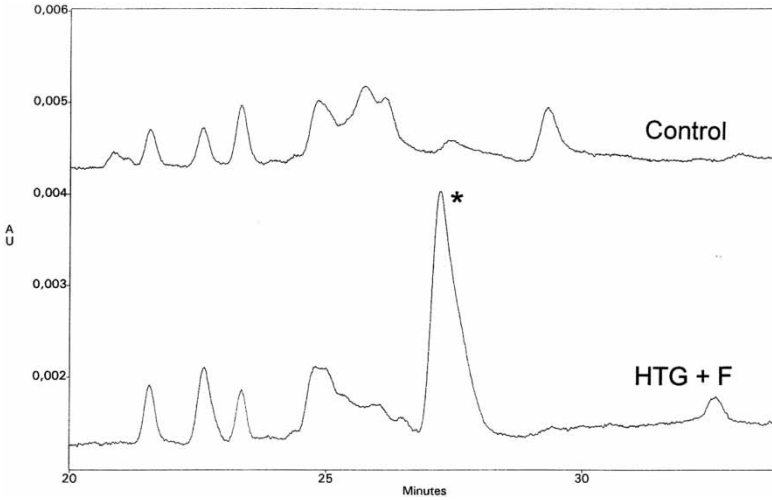


Figure 8. Enlarged sections of the fifth (5) electrophoretic section of the first HPLC fraction (a). An asterisk indicates the presence of a dominant peak in the HTG + F profile.

found in the first HPLC fraction, while the third one occurred in the last (fifth) HPLC fraction. It appears feasible to propose that the first two differences reflect changes in relatively polar peptides, while the last one refers to the differences in rather apolar peptide species. It should be noted, that in

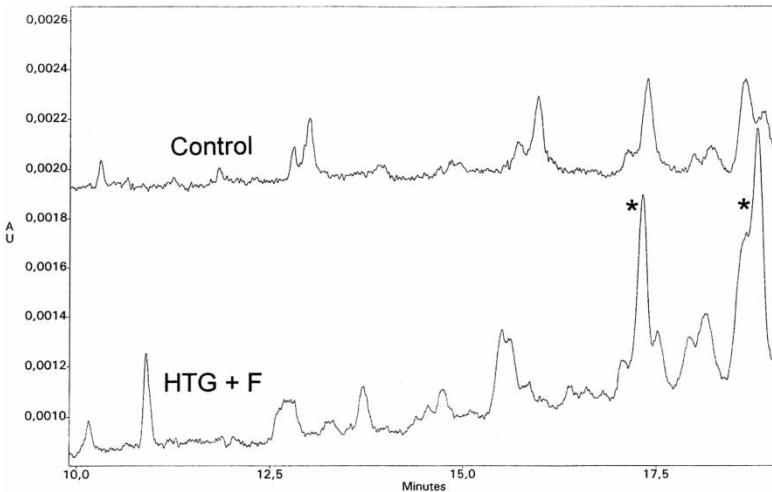


Figure 9. Enlarged sections of the first electrophoretic section (1) of the fifth HPLC fraction (e). Asterisks indicate the dominant peaks in the HTG + F fraction.

Fig. 7 the dominant peak is higher in controls as compared to the HTG + G profile, which indicates that this peptide fraction is involved in the post-translational modification in HTG + F animals. On the other hand, the difference seen in Fig. 9 is indicative of the presence of rather apolar peptide species in the HTG-F samples. Such a situation can arise, e.g., if a lysine containing peptide is reacted with a reactive oxo grouping of the fructose molecule. Because of the complexity of the profiles, the differences found cannot be claimed as the only ones between the control and HTG + F samples. Minor differences regarding other peaks in Figs. 4–6 can be traced, without regard to the abundant number of peptides present in the other fractions and sections, which did not reveal a statistically significant difference between the controls and HTG + F by the statistical approach used.

CONCLUSIONS

By exploiting the complementary nature of HPLC and CE separations of deeply degraded collagenous structures (using *Clostridium histolyticum* collagenase for protein fragmentation), it was possible to reveal several minor, but significant, differences in a very complex set of peptides. These differences result from non-enzymatic post-translational reactions occurring in hypertriglyceridemic animals with simultaneous administration of high sugar diets. It is assumed that these modifications reflect the reaction of free amino groups of the protein with oxo-compounds (fructose and/or fatty acid metabolites). Using linear regression analysis of peptide profiles obtained by sub-sectioning of the electropherograms (used as the second separation step), the presence of three significant modifications (outliers) present in the first and fifth HPLC fractions was confirmed. It is necessary to stress that these may not be the only differences between the profiles compared; however, these are apparently the most distinct ones. Use of a combination of valley-to-valley and common baseline integration of the profiles (profile sections) obtained appears to be a handy tool for revealing differences in complex peptide profiles.

ACKNOWLEDGMENTS

This work was supported by Grant Agency of the Czech Republic, grants Nos. 203/02/1467, 203/03/0716, 304/02/1348, 203/03/D141, and AVOZ 5011 0509.

REFERENCES

1. Cserhati, T.; Forgács, E. Use of Multivariate Mathematical Methods for the Evaluation of Retention Data Matrices. In *Advances in Chromatography*; Brown, P.R., Grushka, E., Eds.; Marcel Dekker Inc.: New York, 1995; Vol. 36, 1–63.

2. Al-Haj, M.A.; Kaliszan, R.; Nasal, A. Test Analytes for Studies of the Molecular Mechanism of Chromatographic Separations by Quantitative Structure-Retention Relationships. *Anal. Chem.* **1999**, *71* (15), 2976–2985.
3. Kaliszan, R.; van Straten, M.A.; Markuszewski, M.; Cramers, C.A.; Claessens, H.A. Molecular mechanism of retention in reversed-phase high-performance liquid chromatography and classification of modern stationary phases by using quantitative structure–retention relationships. *J. Chromatogr. A* **1999**, *855* (2), 455–486.
4. Andrisano, V.; Bertucci, C.; Cavrini, V.; Recanatini, M.; Cavalli, A.; Varoli, L.; Felix, G.; Wainer, I.W. Stereoselective binding of 2,3-substituted 3-hydroxypropionic acids on an immobilised human serum albumin chiral stationary phase: stereochemical characterisation and quantitative structure–retention relationship study. *J. Chromatogr. A* **2000**, *876* (1–2), 75–86.
5. Ivanciuc, O.; Ivanciuc, T.; Cabrol-Bass, D.; Balaban, A.T. Comparison of weighting schemes for molecular graph descriptors: application in quantitative structure–retention relationship models for alkylphenols in gas-liquid chromatography. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 732–743.
6. Acuna-Cueva, R.; Hueso-Urena, F.; Cabeza, N.A.I.; Jimenez-Pulido, S.B.; Moreno-Carretero, M.N.; Martos, J.M.M. Quantitative structure-capillary column gas chromatographic retention time relationships for natural sterols (trimethylsilyl ethers) from olive oil. *J. Am. Chem. Soc.* **2000**, *77*, 627–630.
7. Montana, M.P.; Pappano, N.B.; Debattista, N.B.; Raba, J.; Luco, J.M. High-performance liquid chromatography of chalcones: quantitative structure–retention relationships using partial least-squares (PLS) modeling. *Chromatographia* **2000**, *51*, 727–735.
8. Deyl, Z.; Mikšík, I.; Peptides. *Advanced Chromatographic and Electromigration Methods in BioSciences*, J. Chromatogr. Library; Deyl, Z., Mikšík, I., Tagliaro, F., Tesarová, E., Eds.; Elsevier: Amsterdam, 1998; Vol. 60, Ch. 12, 465–523.
9. Tanaka, S.; Avigad, G.; Eikenberry, E.F.; Brodsky, B. Isolation and partial characterization of collagen chains dimerized by sugar-derived cross-links. *J. Biol. Chem.* **1988**, *263* (33), 17650–17657.
10. Deyl, Z.; Mikšík, I.; Zicha, J. Multicomponent analysis by off-line combination of synchronous fluorescence spectroscopy and capillary electrophoresis of collagen glycation adducts. *J. Chromatogr. A* **1999**, *836* (1), 161–171.
11. Mikšík, I.; Novotná, J.; Uhrová, M.; Jelínková, D.; Deyl, Z. Capillary electrophoresis of large cyanogen bromide peptides of fibre-forming collagens with special reference to cross-linking. *J. Chromatogr. A* **1997**, *772* (1–2), 213–220.
12. Mikšík, I.; Eckhardt, A.; Cserhádi, T.; Forgács, E.; Zicha, J.; Deyl, Z. Evaluation of peptide electropherograms by multivariate mathematical–statistical methods: I. Principal component analysis. *J. Chromatogr. A* **2001**, *921* (1), 81–91.

Received October 28, 2004

Accepted December 4, 2004

Manuscript 6546

