Journal of Chromatography B, 879 (2011) 3102–3107

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

Experimental strategies for the analysis of D-amino acid containing peptides in crustaceans: A review☆

Daniel Soyez[a,b], Jean-Yves Toullec[c], Nicolas Montagné[d], Céline Ollivaux[e,f,g,*]

[a] Université Pierre et Marie Curie – Paris 06, Equipe Biogenèse des signaux peptidiques, ER3, 7 Quai Saint Bernard, F-75251, Paris cedex 05, France
[b] Centre National de la Recherche Scientifique, France
[c] Université Pierre et Marie Curie – Paris 06, UMR 7144 CNRS, Equipe Adaptation et Diversité en Milieu Marin, Station Biologique de Roscoff, F-29682, Roscoff, France
[d] Université Pierre et Marie Curie – Paris 06, UMR A 1272 INRA, Equipe Physiologie de l'Insecte: Signalisation et Communication, F-75251, Paris cedex 05, France
[e] Université Pierre et Marie Curie – Paris 06, UMR 7150 Mer et Santé, Équipe Physiologie Comparée des Erythrocytes, Station Biologique de Roscoff, F-29682, Roscoff, France
[f] Centre National de la Recherche Scientifique, UMR 7150, Station Biologique de Roscoff, France
[g] Université Européenne de Bretagne, UEB, France

ARTICLE INFO

Article history:
Received 13 December 2010
Accepted 17 March 2011
Available online 25 March 2011

Keywords:
Isomerization
D-Amino acid containing peptides
Mass spectrometry
Chromatography
Crustaceans

ABSTRACT

Detection of D-amino acids in natural peptides has been, and remains a challenging task, as peptidyl isomerization is a peculiar and subtle posttranslational modification that does not induce any change in primary sequence or in physicochemical properties of the molecule such as molecular mass or p*I*. Therefore, the presence of a D-amino acid residue in a peptide chain is generally transparent to classical methods of peptide analysis (electrophoresis, chromatography, mass spectrometry, molecular biology). In this article, we will review the various experimental strategies and analytical techniques, which have been used to characterize and to study D-amino acid containing peptides in crustaceans.

Contents

1. Introduction

An old dogma in biology is that proteins are composed of amino acids in the L-configuration exclusively. However in the late 30s, the presence of peptidyl poly-D-glutamic acid in the bacterial cell envelope of the virulent *Bacillus anthracis* was demonstrated [1], and, in the 40s, a rapidly increasing number of microbial peptide antibiotics containing frequently unusual amino acid residues and notably D-amino acids (D-AAs) were discovered (review in [2]). Natural occurrence of D-AAs in proteins was rather considered as a peculiarity of microorganisms. However, D-AA residues were later found in proteins from animals, including Man. For example, D-Asp has been found in several human proteins, such as dentin [3], α-crystalline from lens of patients with cataract [4], and β-amyloid peptide from brains of Alzheimer's patients [5]. In these long-living proteins, the origin of peptidyl D-AAs may be explained by non-enzymatic racemization and isomerization associated with aging or diseases [6].

☆ This paper is part of the special issue "Analysis and Biological Relevance of D-Amino Acids and Related Compounds", Kenji Hamase (Guest Editor).

* Corresponding author at: Université Pierre et Marie Curie – Paris 06, UMR 7150 Mer et Santé, Équipe Physiologie Comparée des Erythrocytes, Station Biologique de Roscoff, F-29682, Roscoff, France. Tel.: +33 2 98 29 25 53; fax: +33 2 98 29 23 10.
E-mail address: celine.ollivaux@upmc.fr (C. Ollivaux).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.03.032

Table 1
Amino acid sequence of Crustacean Hyperglycaemic Hormone (CHH) and Vitellogenesis Inhibiting Hormone (VIH) isoforms, and sequences of synthetic peptides used in the different studies, as standards for chromatography or immunogens for antibodies production. D-Residues are in bold letter. Cysteyl residues (underlined) of CHH and VIH are always paired as follows: 1–5, 2–4, 3–6.

Peptide name	Sequence
CHH A	pEVFDQACKGVYDRNLFKKLDRVCEDCYNLYRKPFVATTCRENCYSNWVFRQCLDDLLLSDVIDEYVSNVQMVNH2
DPhe$_3$CHH A	pEV**F**DQACKGVY...
Oct-L	pEVFDQACK
Oct-DF$_3$	pEV**F**DQACK
VIH	ASAWFTNDECPGVMGNRDLYEKVAWVCNDCANIFRNNDVGVMCKKDCFHTMDFLWCVYATERHGEIDQFRKWVSILR
DTrp$_4$VIH	ASA**W**FTNDECPGVM...
Hep-L	ASAWFTN
Hep-D	A**S**AWFTN
S$_2$Hep-D	AS**A**WFTN
A$_3$Hep-D	ASA**W**FTN
W$_4$Hep-DF$_5$	ASAW**F**TN
Dec-L	ASAWFTNDEC
Dec-DW$_4$	ASA**W**FTNDEC

In another context, a D-Ala residue was reported to be present in an opioid peptide from skin secretion of the tree frog *Phyllomedusa sauvagei* [7]. At the time of publication, the scientific community was quite doubtful about this work, which was yet a real breakthrough in peptide studies. Since, D-AA residues of different nature have been found in bioactive peptides from venom or nervous tissue from various species belonging to molluscs, arachnids, crustaceans and vertebrates (platypus and frogs) (review in [8]). Only one D-AA was found in the peptide chain, and close to one end, most frequently the N-terminus. Moreover, a classical codon has always been found in the mRNA in the position where the D-residue is present in the mature peptide [9].

Several hypotheses may be formulated to explain the presence of a D-residue in the peptide chain. It may result from different mechanisms such as conversion of a free L-AA into its D-counterpart before its incorporation, or enzymatic posttranslational modification of an L-residue after the peptide chain synthesis. This is the case of the crustacean neuropeptides that have been studied in our laboratory for many years. Indeed, Crustacean Hyperglycaemic Hormone (CHH) and Vitellogenesis Inhibiting Hormone (VIH) are 70- to 80-residue neuropeptides, depending of the peptide and of the considered species. They exhibit hormonal activities and regulate energetic metabolism and reproduction, respectively. CHH constitutes the archetype of a peptide family present in Arthropods. This family is mostly characterized by six cysteyl residues at conserved positions, paired in three-disulfide bridges (see Table 1). CHH and VIH were shown to be present in the major neuroendocrine organ of the lobster *Homarus americanus*, the X organ-sinus gland complex, as two isomers differing by the change of a specific residue from the L- to the D-configuration. This change results in modifications of the biological activity of the peptide [10].

In the course of studies of these peptides, a number of different analytical methods have been utilized and the following text describes the strategies developed. To conclude, focus will be on an approach seldom made in this field, which is the study of D-amino acid containing peptides (DAACPs) at the cellular level, by immunohisto- and immuncytochemistry.

2. Different hydrophobicity in RP-HPLC for L- and D-amino acid containing peptides from crustaceans: peptide mapping and chiral amino acid analysis

As numerous discoveries in Life Sciences, the discovery of DAACPs in crustaceans has relied on the anecdotic observation, in the early 90s, that CHH and VIH from neuroendocrine glands of the lobster *Homarus americanus* were eluted from C-18 RP-HPLC (reverse phase – high performance liquid chromatography) columns as pairs of peaks composed of peptides with the same molecular mass, p*I*, amino acid composition and N-terminal sequence [11–13] (Fig. 1). It was then proposed that these isoforms may differ by the pairing of cysteyl residues involved in the three-disulfide bridges but this hypothesis was not confirmed by later studies, as described below.

As a matter of fact, a decisive clue in the elucidation of the difference between the CHH isoforms of one pair was that comparative peptide mapping by RP-HPLC after cleavage with endoproteinase Lys-C revealed identical fragments except one. This fragment was eluted with a different retention time depending on the CHH isoform considered, but in both cases, an identical molecular mass corresponding to the N-terminal octapeptide was determined by ESI MS (electrospray ionization mass spectrometry). As it was known from previous studies that the amino acid sequences of both isoforms were identical, a likely explanation was a modification of the chirality of one or several residues in the N-terminal fragment of CHH [14]. Then, a chiral amino acid analysis was performed to verify this hypothesis. The method used was based on [15], with an acid hydrolysis of the peptide followed by a pre-column derivatization of amino acids with the chiral reagent, 1-fluoro-2,4-dinitrophenyl-5-L-alanine (Marfey's reagent) and subsequent chromatographic separation on an analytical C-18 column with a detection of the derivatives at 340 nm. Identification of the derivatives was made by comparison with similar analyses of L- and D-amino acid standard solutions (Fig. 2).

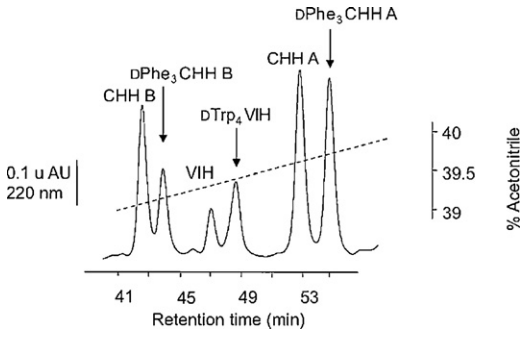

Fig. 1. RP-HPLC profile of an acetic acid extract of lobster *Homarus americanus* sinus glands. Only the part of the chromatogram where CHHs and VIHs are eluted is shown Peptides from 30 lobster sinus glands were extracted with 10% acetic acid and fractionated on a Kromasil C18 column (3.5 μm particle size, 250 mm × 4.6 mm internal diameter, AIT Chromato, Le Mesnil-le-roi, France). Peptides were eluted by a linear gradient of acetonitrile in water at a flow-rate of 0.75 mL/min (dotted line). Both solvents contained trifluoroacetic acid (0.1% in water and 0.08% in acetonitrile). The ultraviolet absorbance of the eluent was monitored at 220 nm.
Modified from [33].

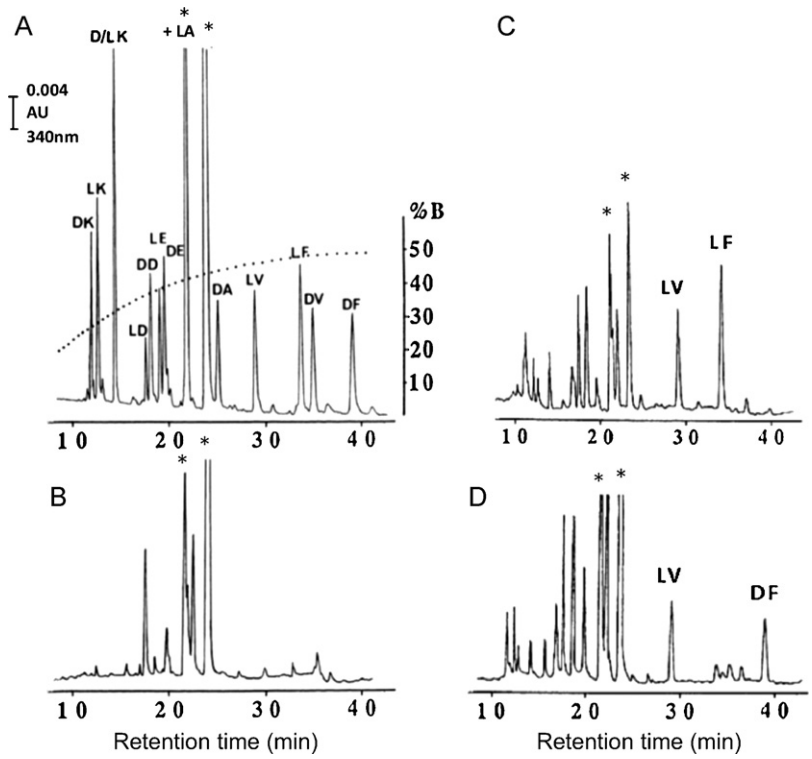

Fig. 2. Chiral amino acid analysis was performed by pre-column derivatization with the chiral reagent,1-fluoro-2,4-dinitrophenyl-5-L-alanineamide (Marfey's reagent) according to [15]. Chromatographic conditions were as follows. The column filled with Nucleosil C-18 (5 μm particle size, 250 mm × 4.6 mm internal diameter, Macherey-Nagel, Düren, Germany). Solvent A was triethylamine/phosphoric acid buffer (40 mM, pH 2.2); solvent B was acetonitrile/2-propanol (4/1). Elution was realized by a convex gradient (dotted line in A) from 20% to 50% B at a flow rate of 1 mL/min. UV absorbance was monitored at 340 nm. All samples (including standard amino acid solutions and blank) have been subjected to acid hydrolysis before chiral analysis. (A) RP-HPLC separation of derivatives of L- and D-amino acid standards (one-letter code). *: reagent peaks. Note that each lysine isomer is eluted, after derivatization, as two peaks, one with identical RT for both isomers (labelled D/L K on the graph) and the other with a different RT according to the isomer (DK and LK). (B) similar analysis of blank sample. (C) analysis of 250 pmol of acid hydrolysate of the N-terminal fragment from the more polar CHHA isoform. (D) Analysis of 250 pmol of acid hydrolysate of the N-terminal fragment from the less polar CHHA isoform of lobster *Homarus americanus*. Modified from [14].

This protocol has allowed demonstrating the presence of an L-Phe in the hydrolysate from the N-terminal octapeptide of the most polar isoform and a D-Phe in the fragment of the most hydrophobic isoform (Fig. 2). $DPhe_3$ CHHA and $LPhe_3$ CHHA (simply named CHHA) are both hyperglycaemic hormones but exhibited differential activities in the time course of glycaemia [14]. In subsequent studies, the double peaks of CHHB were also identified as $DPhe_3$ CHHB and CHHB [16] (Fig. 1).

Since several decades, numerous protocols have been described in the literature, which include derivatization of amino acids with chiral reagent followed by chromatography (review in [17]). This approach, though technically not very straightforward and which needs nanomolar amounts of peptide (review in [18]), still remains the most accurate and reliable method to determine the stereochemistry of AA residues in peptides.

3. Identical N-terminal sequences for DAACPs of different species: RP-HPLC and ELISA with specific antibodies

Once the nature of the D-residue in CHH has been established in the American lobster by the analytical methods described above, it was decided to design specific tools, namely specific antibodies, to search for similar peptides in other species and to study the production site in crayfishes and lobsters.

For that sake, conformational antibodies have been developed against synthetic peptides with sequences corresponding to the N-terminal octapeptides of L- and $DPhe_3$ CHH. Synthetic peptides, oct-L and oct-DF_3 (Table 1), were coupled to bovine thyroglobulin, an immunogenic carrier protein absent in crustacean peptide mixture [16]. The complexes thyroglobulin-oct-L and thyroglobulin-oct-DF_3 were injected into rabbits. At the end of the immunization process, it was observed that the titre of specific IgGs was higher in sera from rabbits injected with the DAACP (rb-anti-pQD) than in those from animals injected with the all L-peptide (rb-anti-pQL) (personal observation). The polyclonal antibodies obtained exhibited a high specificity [16,19]. These antisera were used in metabolic studies [20] and to look for $DPhe_3$ CHH in HPLC fractions of sinus gland extracts from different crayfish and lobster species, where CHH was known to have the same N-terminus sequence as in American lobster (Table 2). In other species where CHH exhibited a different N-terminal sequence (such as shrimps or crabs), no cross-immunoreactivity was observed (unpublished results).

In addition, these antibodies and others produced similarly in guinea pigs were used in immunohistochemistry (IHC) to localize peptides in the producing organs, notably at the cellular level, as described later.

4. Peptide mapping, comparison with synthetic peptides and validation with specific antibodies: the example of VIHs

In DAACPs characterized in animals so far, the D-residue is found most frequently near the N-terminus (until residue 4). A few years ago, a new approach founded on this peculiarity was developed to study VIH isoforms of the American lobster. The stereoisomers (named VIH I and VIH II since 1991) were characterized by an approach combining complementary techniques of bio- and immuno-chemistry [21].

Table 2
Techniques used for detection of a D-residue in crustacean VIH and CHH Enzymes used for peptide mapping: (1) endoproteinase Lys-C, (2) lysyl endopeptidase (3) trypsin, (4) endoproteinase Asp-N. Specific antibodies against N-terminus of peptides: SA. Synthetic peptides: SP. ESI-Q MS: Electrospray Ionization-Quadrupole Mass Spectrometry. MALDI-TOF MS: Matrix Assisted Laser Desorption Ionization-Time Of Flight Mass Spectrometry.

Peptide name	Technique	Reference
Hoa DPhe$_3$ CHH	Peptide mapping (1)/ESI Q MS/Chiral AA analysis	[14]
Prc DPhe$_3$ CHH	Peptide mapping (2)/Chiral AA analysis	[42]
Prb DPhe$_3$ CHH	ELISA (SA)	[43]
Orl DPhe$_3$ CHH	ELISA (SA)	[16]
Chd DPhe$_3$ CHH	Peptide mapping (3)/MALDI-TOF MS/Chiral AA analysis/ELISA (SA)	[44]
Asl DPhe$_3$ CHH	ELISA (SA)	[45]
Hog DPhe$_3$ CHH	ELISA (SA)	[21]
Nen DPhe$_3$ CHH	ELISA (SA)	Unpublished results
Hoa DTrp$_4$ VIH	peptide mapping (4)/MALDI-TOF MS/SP	[33]
Hog DTrp$_4$ VIH	ELISA (SA)	[21]
Nen DTrp$_4$ VIH	ELISA (SA)	Unpublished results

Hoa: *Homarus americanus*; *Prc*: *Procambarus clarkii*; *Prb*: *Procambarus bouvieri*; *Orl*: *Orconectes limosus*; *Chd*: *Cherax destructor*; *Asl*: *Astacus leptodactylus*; *Hog*: *Homarus gammarus*; *Nen*: *Nephrops norvegicus*.

In a first step, retention times in RP-HPLC were compared between (i) a panel of synthetic heptapeptides corresponding to the primary sequence of the N-terminal VIH (ASAWFTN) with an all L-heptapeptide (Hep-L) or with a D-residue in a various position (HepDS$_2$, HepDA$_3$, HepDW$_4$, or HepDF$_5$; Table 1) and (ii) VIH I and II digests generated by endoproteinase Asp-N. Results (Fig. 3) suggested that a D-Trp was present in position 4 of the VIH II sequence. In this study, the mass of the fragments and synthetic peptides were verified by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. It is worth noting that the standard peptides with a D-residue in a various position exhibited different hydrophobicity values, agreeing with previous results [22].

Once D-Trp$_4$ has been identified as the putative D-residue in VIH II (named DTrp$_4$ VIH), polyclonal antibodies were produced in guinea pigs against synthetic decapeptides designed from the sequences of the N-terminus of VIH with either a L- or a D-Trp in position 4 (Dec-L and Dec-DW4 in Table 1). As in the case of rb-anti-pQD (raised against the N-terminus of DPhe$_3$ CHH), gp-anti-DW$_4$ (recognizing DTrp$_4$ VIH) displayed a very high specificity level. By contrast, the antiserum produced against the N-terminus of VIH exhibited a strong cross reactivity with Dec-DW$_4$. Consequently, it was necessary to purify the specific IgGs by affinity chromatography before ELISA and immunohistochemistry. Then, the demonstration by direct ELISA of the reactivity of VIHs with antisera developed against decapeptides containing an L- or a D-Trp respectively, confirmed unambiguously the presence of VIH and DTrp$_4$ VIH in lobster.

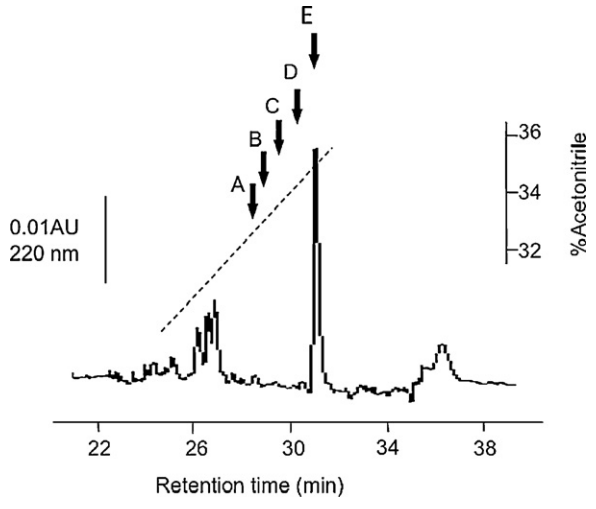

Fig. 3. RP-HPLC profile of a VIH II digest with endo-Lys C (10 lobster *Homarus americanus* sinus glands equivalents). Only the part of the chromatogram where peptides of interest are eluted is shown. The letters above the chromatogram indicate the elution position of the different synthetic peptides (sequences in Table 1) in identical chromatographic conditions. A: Hept-L, B: Hep-DS$_2$, C: Hep-DA$_3$, D: Hep-DF$_5$, E: Hep-DW$_4$. RP-HPLC analyses were made on a narrow bore Nucleosil C18 column (5 μm particle size, 250 mm × 2 mm internal diameter, Macherey-Nagel, Düren, Germany). Peptides were eluted from the column by a gradient of acetonitrile in water at a flow-rate of 0.2 mL/min (dotted line). Both solvents contained trifluoroacetic acid (0.1% in water and 0.08% in acetonitrile). Elution was monitored by UV detection at 220 nm.
Modified from [33].

5. D-Amino acid detection and quantification in crustacean peptides by mass spectrometry

In initial studies described above, mass spectrometry (MS) has been utilized as an analytical tool to determine the mass of neuropeptides or fragments, the detection of D-residues relying on chiral amino acid analysis or/and recognition by specific conformational antibodies (Table 2). However, MS has been applied for several years to differentiate all-L peptides from those containing a D-residue. This was made possible because the presence of a D-residue induces a difference in the fragmentation pattern upon analysis [23]. Until our work described in the next paragraph, this phenomenon has been mostly analyzed after ionization of peptides by electrospray, such as in [24].

To develop new tools for characterization of DAACPs, another mode of ionization, which is robust and widely available in research laboratories, namely MALDI coupled to tandem mass spectrometry (TOF–TOF), was applied to synthetic peptides mentioned above (Table 1). We have studied the incidence of the position of the D-residue and of the peptide length on fragmentation pattern induced through metastable decomposition or through collision-activated dissociation [25]. Discrimination between isomers and their semi-quantification were performed by comparing the intensity ratios and the abundance ratios of fragment ions observed in the MS/MS spectra of the corresponding $(M+H)^+$ ion generated from LAACPs and DAACPs (Fig. 4). Interestingly, relevant fragmentation patterns could be correlated to the position of the D-residue in the sequence, therefore, the distribution of the abundance ratios could be considered as a relevant fingerprint of the different isomers. This report [25] shows that MALDI constitutes an efficient method for distinguishing and quantifying DAACPs. Nevertheless, the article depicts also possible artefacts and limitations due to sodium-cationization, which was observed preferentially for one particular isomer and not for another one.

On the overall, MALDI TOF-TOF appears as a robust and fast technique, which may be adapted for systematic searches of DAACPs in various biological models. This approach could also be used to look

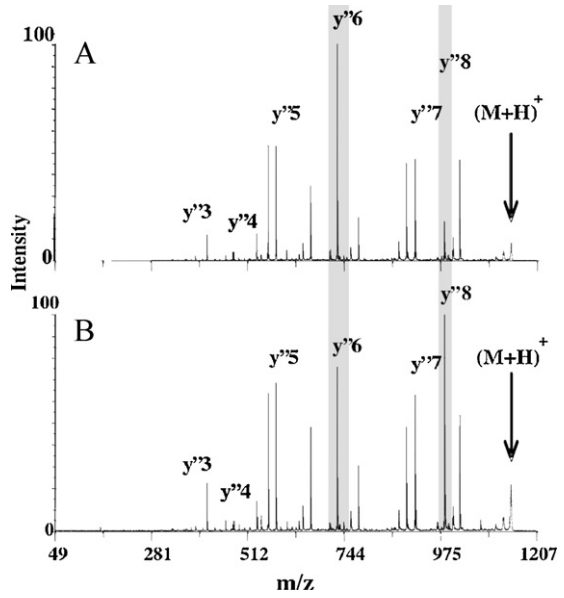

Fig. 4. Typical MALDI-TOF-TOF mass spectra (metastable decomposition) of the protonated molecules $(M+H)^+$ of the decapeptides Dec-L (A), and Dec-DW_4 (B). Fragment ions of the y″ series are noted on the spectra. The grey areas correspond to the regions of interest in which the relative intensities of the ions vary.
Modified from [25].

for peptide isomers at the cellular level, as it was performed to characterize different cell types from the search of a short peptidic sequence [26].

6. D-Amino acid containing peptides detection by immunohisto/cyto-chemistry

As described above, immunoassays (ELISA) using specific and polyclonal antibodies against the N-terminus of CHH and VIH stereoisomers have allowed detecting DAACPs in various crustacean species. Otherwise, DAACPs quantification by a combination of indirect ELISA (double sandwich) and RP-HPLC was described in [27].

In the literature, besides works on crustacean peptides detailed below, a very few studies of DAACPs at the cellular and subcellular levels have been reported. Two of them dealt with the localization of L- and D-Ala_2deltorphin in cutaneous serous gland of the south American frog *Phyllomedusa sauvegei* [28,29] and, in more recent works, the occurrence of proteins containing D-β-aspartyl residues in various tissues (lens, skin, cardiac muscle. . .) of elderly humans was described [30,31].

By contrast, localization of CHH and VIH isomers within tissues and cells of the crustacean neuroendocrine system have been especially well documented in several immunohisto- and immunocyto-chemical investigations using the specific antisera described above. The first study on peptide isomers at the cellular level conducted by immunohistochemistry was reported in [16]. In this work, use of the specific conformational antibodies on paraffin sections of neuroendocrine organs from the crayfish *Orconectes limosus* allowed the first demonstration of a cell-specificity for this peculiar posttranslational modification. Indeed, in the X organ, constituted by neuronendocrine pericarya producing CHH and VIH, every hyperglycaemic hormone-containing cell was labelled with the gp-anti-pQL antiserum while only a few of them were visualized with the rb-anti-pQD antiserum.

In a next study [19], confocal analysis of whole-mounts of X organ-sinus gland complexes of *O. limosus* was performed after double immunofluorescence labelling with the specific antibodies. It was confirmed that the number of the different CHH cells was constant, with a figure of eight $DPhe_3$ CHH containing cells over a total of 30 CHH cells. Moreover, it was observed that the extent of Phe_3isomerization increases from the pericarya to the axonal arborization of the specialized neurosecretory cells, probably as a result of the activity of a putative enzyme (a peptidylaminoacyl L/D isomerase) confined within the secretory granules.

This work was later completed by ultrastructural immunocytochemistry (ICC) using immunogold labelling on crayfish neuroendocrine organs and quantification by image analysis [32]. It was confirmed that isomerization occurs within the secretory granules and progresses as the granules migrate along the axonal tract. Interestingly, quantification of CHH isomers in $DPhe_3$ CHH-producing cells has demonstrated that not all the pool of CHH synthesized is isomerized. In addition, the great variability in the proportion of L- and D-immunoreactivity in granules in every cell region has suggested a very heterogeneous distribution of the putative isomerase within the secretory pathway.

More recently, IHC/ICC using confocal and electron microscopy, respectively, were applied to both CHH and VIH in lobster X organ, using a battery of different conformational antibodies. An important observation was that $DPhe_3$ CHH and $DTrp_4$ VIH are not only synthesized in the same cells but are co-packaged within the same secretory granules [33].

7. Conclusion – futures investigations

To conclude, other analytical approaches may be used to identify and to analyze DAACPs. For example, Nuclear Magnetic Resonance (NMR) experiments have been performed to confirm the presence of a D-residue in two platypus toxins, C-type natriuretic peptide (D-Leu_2 for OvCNPb) and defensin-like peptide (D-Met_2 for DLP-2) [34], [35]. In NMR experiments on the conotoxin peptide, conomorphin, it was demonstrated the $DPhe_{13}$ is necessary to form a tight loop in the middle of the peptide, as this loop was not present in the L-counterpart of the toxin [36].

An alternative way to the direct characterization of a D-residue in a peptide sequence consists in searching for the putative enzymes responsible for the posttranslational epimerization, the peptide-isomerases. To date, amino acid sequences of such enzymes have been obtained from spider venom [37] and frog skin secretion [38]. Surprisingly enough, they appear totally unrelated, with no similarity between the two sequences. Indeed, the frog enzyme exhibits similarities with the N-terminal H-domain of human IgG-Fc binding protein, whereas the spider isomerase appears to belong to the serine protease family. A peptide-isomerase has also been isolated from the venom of platypus, but its amino acid sequence remains unknown [39]. Nevertheless, the sequencing of platypus venom transcriptome may provide a powerful tool to uncover isomerase(s) displaying sequence identity with previously identified enzymes [40]. Obviously, the large structural and functional divergences between these enzymes impede, for instance, any speculation about the characteristics of the putative crustacean isomerase(s). A break in this field of research come from the demonstration of a putative isomerase activity in mouse heart [41], and this result opens fascinating perspectives to find DAACPs and unravel their possible role in Mammals and especially in Man.

References

[1] G. Ivanovics, V. Bruckner, Die Naturwiss. 25 (1937) 250.
[2] M. Bodanszky, D. Perlman, Nature 218 (1968) 291.
[3] P.M. Helfman, J.L. Bada, Proc. Natl. Acad. Sci. U.S.A. 72 (1975) 2891.
[4] N. Fujii, K. Satoh, K. Harada, Y. Ishibashi, J. Biochem. 116 (1994) 663.

[5] A.E. Roher, J.D. Lowenson, S. Clarke, C. Wolkow, R. Wang, R.J. Cotter, I.M. Reardon, H.A. Zurcher-Neely, R.L. Heinrikson, M.J. Ball, et al., J. Biol. Chem. 268 (1993) 3072.
[6] N. Fujii, Y. Momose, N. Ishii, M. Takita, M. Akaboshi, M. Kodama, Mech. Ageing Dev. 107 (1999) 347.
[7] P.C. Montecucchi, R. De Castiglione, S. Piani, L. Gozzini, V.R. Erspamer, Int. J. Peptide Protein Res. 17 (1981) 275.
[8] G. Kreil, Annu. Rev. Biochem. 66 (1997) 337.
[9] A. Mor, M. Amiche, P. Nicolas, Trends Biochem. Sci. 17 (1992) 481.
[10] D. Soyez, in: M.F.a.R. Nagabhushanam, (Ed.), Recent Advances in Marine Biotechnology, Science Publishers, Enfield (NH) USA, Plymouth, UK, 2003, p. 280.
[11] D. Soyez, P.Y. Noel, J.E. Van Deijnen, M. Martin, A. Morel, G.G. Payen, Gen. Comp. Endocrinol. 76 (1990) 261.
[12] C.P. Tensen, K.P.C. Janssen, D. Soyez, F. Van Herp, Peptides 12 (1991) 241.
[13] D. Soyez, J.P. Lecaer, P.Y. Noel, J. Rossier, Neuropeptides 20 (1991) 25.
[14] D. Soyez, F. Van Herp, J. Rossier, J.P. Le Caer, C.P. Tensen, R. Lafont, J. Biol. Chem. 269 (1994) 18295.
[15] A. Scaloni, M. Simmaco, F. Bossa, Anal. Biochem. 197 (1991) 305.
[16] D. Soyez, A.M. Laverdure, J. Kallen, F. Van Herp, Neuroscience 82 (1998) 935.
[17] L. Bai, S. Sheeley, J.V. Sweedler, Bioanal. Rev. 1 (2009) 7.
[18] T. Lida, T. Santa, A. Toriba, K. Imai, Biomed. Chromatogr. 15 (2001) 319.
[19] D. Soyez, J.Y. Toullec, C. Ollivaux, G. Geraud, J. Biol. Chem. 275 (2000) 37870.
[20] C. Ollivaux, D. Soyez, Eur. J. Biochem. 267 (2000) 5106.
[21] C. Ollivaux, J. Vinh, D. Soyez, J.Y. Toullec, FEBS J. 273 (2006) 2151.
[22] T. Munegumi, Chem. Biodivers. 7 (2010) 1670.
[23] C.M. Adams, F. Kjeldsen, R.A. Zubarev, B.A. Budnik, K.F. Haselmann, J. Am. Soc. Mass Spectrom. 15 (2004) 1087.
[24] S.V. Serafin, R. Maranan, K. Zhang, T.H. Morton, Anal. Chem. 77 (2005) 5480.
[25] E. Sachon, G. Clodic, C. Galanth, M. Amiche, C. Ollivaux, D. Soyez, G. Bolbach, Anal. Chem. 81 (2009) 4389.
[26] V. Redeker, J.Y. Toullec, J. Vinh, J. Rossier, D. Soyez, Anal. Chem. 70 (1998) 1805.
[27] C. Ollivaux, H. Dircksen, J.Y. Toullec, D. Soyez, J. Comp. Neurol. 444 (2002) 1.
[28] C. Lacombe, C. Cifuentes-Diaz, I. Dunia, M. Auber-Thomay, P. Nicolas, M. Amiche, Eur. J. Cell Biol. 79 (2000) 631.
[29] C. Auvynet, N. Seddiki, I. Dunia, P. Nicolas, M. Amiche, C. Lacombe, Eur. J. Cell Biol. 85 (2006) 25.
[30] T. Kinouchi, N. Fujii, Chem. Biodivers. 7 (2010) 1403.
[31] N. Fujii, Y. Kaji, T. Nakamura, R. Motoie, Y. Mori, T. Kinouchi, Chem. Biodivers. 7 (2010) 1389.
[32] D. Gallois, M.J. Brisorgueil, M. Conrath, P. Mailly, D. Soyez, Eur. J. Cell Biol. 82 (2003) 431.
[33] C. Ollivaux, D. Gallois, M. Amiche, M. Boscaméric, D. Soyez, FEBS J. 276 (2009) 4790.
[34] A.M. Torres, I. Menz, P.F. Alewood, P. Bansal, J. Lahnstein, C.H. Gallagher, P.W. Kuchel, FEBS Lett. 524 (2002) 172.
[35] A.M. Torres, C. Tsampazi, D.P. Geraghty, P.S. Bansal, P.F. Alewood, P.W. Kuchel, Biochem. J. 391 (2005) 215.
[36] Y. Han, F. Huang, H. Jiang, L. Liu, Q. Wang, Y. Wang, X. Shao, C. Chi, W. Du, C. Wang, FEBS J. 275 (2008) 1976.
[37] Y. Shikata, T. Watanabe, T. Teramoto, A. Inoue, Y. Kawakami, K. Nishizawa, K. Katayama, M. Kuwada, J. Biol. Chem. 270 (1995) 16719.
[38] A. Jilek, C. Mollay, C. Tippelt, J. Grassi, G. Mignogna, J. Mullegger, V. Sander, C. Fehrer, D. Barra, G. Kreil, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 4235.
[39] A.M. Torres, M. Tsampazi, E.C. Kennett, K. Belov, D.P. Geraghty, P.S. Bansal, P.F. Alewood, P.W. Kuchel, Amino Acids 32 (2007) 63.
[40] C.M. Whittington, J.M. Koh, W.C. Warren, A.T. Papenfuss, A.M. Torres, P.W. Kuchel, K. Belov, J. Proteomics 72 (2009) 155.
[41] J.M. Koh, S.J. Chow, B. Crossett, P.W. Kuchel, Chem. Biodivers. 7 (2010) 1603.
[42] A. Yasuda, Y. Yasuda, T. Fujita, Y. Naya, Gen. Comp. Endocrinol. 95 (1994) 387.
[43] M.B. Aguilar, D. Soyez, R. Falchetto, D. Arnott, J. Shabanowitz, D. Hunt, A. Huberman, Peptides 16 (1995) 1375.
[44] P. Bulau, I. Meisen, B. Reichwein-Roderburg, J. Peter-Katalinic, R. Keller, Peptides 24 (2003) 1871.
[45] L. Serrano, G. Charmantier, D. Soyez, E. Grousset, C. Spanings-Pierrot, J. Exp. Biol. 206 (2003) 979.