CD and FTIR Spectroscopic Studies of Carbohydrate-modified Opioid Peptides

Elemér Vass,a Miklós Hollósi,a Štefica Horvat,b and Maja Roščič,b,*

aDepartment of Organic Chemistry, Institute of Chemistry, Eötvös Loránd University, P. O. Box 32, H-1518 Budapest 112, Hungary

bRuđer Bošković Institute, Division of Organic Chemistry and Biochemistry, Laboratory for Carbohydrate, Peptide and Glycopeptide Research, P. O. Box 180, 10002 Zagreb, Croatia

RECEIVED OCTOBER 23, 2007; REVISED MARCH 20, 2008; ACCEPTED APRIL 29, 2008

The variety of vital functions played by glycoproteins in many physiological and pathological processes inspired the design of glycopeptides and the study of the effect of glycosylation on conformation. This work reports comparative circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopic studies on linear and cyclic Amadori and imidazolidinone-type glycopeptides 3–8 in comparison with spectroscopic data of the non-modified flexible parent peptides, Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH, 1) and its amide (H-Tyr-Gly-Gly-Phe-Leu-NH₂, 2). The CD and FTIR measurements were performed in different solvents in order to expose the structural and conformational differences caused by a keto-sugar, a rigid 5-membered imidazolidinone ring and/or cyclization. The combined application of CD and FTIR spectroscopy allowed to evaluate the relative amount of the extended and folded conformers of Amadori as well as imidazolidinone compounds and proved to be an effective tool for characterization of their structural features leading to a deeper understanding of their conformational behaviour.

Keywords

glycopeptides
Amadori
imidazolidinone
conformational analysis
circular dichroism
IR spectroscopy

INTRODUCTION

Opioid peptides are responsible for a variety of processes in organisms, the best characterized of which are analgesia, sedation, euphoria, respiratory depression, and a peripheral action as an antidiarrheic.1 From medicinal chemical viewpoint, a very broad spectrum of biological activities render endogenous opioid peptides, Leu- and Met-enkephalin (Tyr-Gly-Gly-Phe-Leu/Met),2,3 as important targets with a good potential for further development as therapeutic agents. Because of the very poor metabolic stability of biologically active peptides, numerous analogs have been designed to overcome pharmaceutical and pharmacokinetic barriers in clinical drug application.4–7

The carbohydrate moieties of glycoproteins and glycopeptides are believed to have important roles in their biological activity.5,9 Among these, the structural effect on a peptide backbone represents a major function.10,11 However, the issue of how glycosylation affects protein or peptide structure is unclear. It was reported that carbohydrate moiety aids in the formation of a secondary structure of a peptide backbone, especially the β-turn,12,13 or stabilizes the peptide backbone conformation.14,15 In
some cases no structural change is evident, and in some cases the original α-helical structure was found to be broken, or perturbed.

This work reports the comparative circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopic properties of glycopeptides 3–8 in comparison with spectroscopic data obtained for the non-modified parent opioid peptides, Leu-enkephalin (1) and Leu-enkephalin amide (2) (Figure 1). One group of investigated compounds are Amadori compounds 3 and 4 in which the

Figure 1. Structures of compounds 1–8: Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu, 1) and its amide (Tyr-Gly-Gly-Phe-Leu-NH₂, 2) as well as their glycoconjugates, Amadori compounds 3–5 and imidazolidinones 6–8.

linear peptide is alkylated at the N-terminal position by a 1-deoxy-D-fructose unit. Adduct 5, the cyclic analog of compound 3, possesses an ester bond between the C-6 hydroxy group of the sugar moiety and the C-terminal carboxy group of the peptide, 1-deoxy-D-fructofuranose acting as a bridge between the Leu-enkephalin terminal parts. The rigid 5-membered imidazolidinone ring is characteristic of the second class of compounds. In these adducts an imidazolidinone moiety connects the acyclic sugar residue with the linear peptide chain (6, 7). The imidazolidinone compounds 6 with D-glucopyranosyl pen-titol structures are diastereoisomers with trans (6a) and cis (6b) relative geometry of the carbon substituents at the imidazolidinone ring moiety. In the corresponding bicyclic imidazolidinone analogs 8a and 8b, a 19-membered ring is formed through an ester bond between the primary hydroxyl group of the D-glucopyranosylpentitol residue and the C-terminus of the peptide.

The measurements were performed in different solvents in order to expose the structural and conformational differences caused by a keto-sugar, a rigid 5-membered imidazolidinone ring and/or cyclization.

**EXPERIMENTAL**

**Glycopeptide Preparation**

Leu-enkephalin (1) and Leu-enkephalin amide (2) were purchased from Bachem. Amadori compounds, N-[1-deoxy-D-fructos-1-yl]-L-tyrosylglycyl-L-phenylalanyl-L-leucine (3) and cyclo-{{N-[6]-1-deoxy-α,β-D-fructofuranos-1-yl}L-tyrosylglycyl-L-phenylalanyl-L-leucyl-(1→O)} (5) were synthesized under the conditions described by Horvat et al. Imidazolidinone derivatives, N-{{[2-(D-glucose-penti-tol-1-yl)-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl]}acyetylglycyl-L-phenylalanyl-L-leucine (6a, trans isomer; 6b, cis isomer) and cyclo-{{N-[2-[5]-D-glucose-pentitol-1-yl]-4-(4-hydroxybenzyl)-5-oxoimidazolidin-1-yl-(1→O)}acyetylglycyl-L-phenylalanyl-L-leucyl} (8a, trans isomer; 8b, cis isomer) were prepared according to the reported procedure. All compounds with the exception of 4 and 5 were desalted using an octadecylsilica solid-phase extraction (SPE) cartridge (500 mg, 2.8 mL).

**CD Measurements**

Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectrometer at room temperature. A quartz cuvette with 0.2 mm path length was used for far-UV (250–185 nm) measurements and a quartz cuvette with 10.0 mm path length for near-UV (310–250 nm) measurements. CD spectra were taken in 2,2,2-trifluoroethanol (TFE; Aldrich, NMR grade), water and in 1:1 mixture of these solvents. The sample concentrations ranged between 0.2–0.4 mg/mL.

**FTIR Measurements**

FTIR spectra were recorded on a Bruker Equinox 55 spectrometer at room temperature in a CaF2 cell of 0.2 mm path length. The sample concentration in TFE was 2 mg/mL in each measurement.

---

**TABLE I.** CD spectral parameters, molar CD (Δε/(dm²mol⁻¹cm⁻¹)) and mean residue ellipticity [θ]ₘᵣ (deg cm² dmol⁻¹) of compounds 1–5 in TFE, TFE-water (1:1) mixture and water.

<table>
<thead>
<tr>
<th>Compound</th>
<th>TFE</th>
<th>TFE/H₂O = 1/1</th>
<th>H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>191.5</td>
<td>7.37</td>
<td>4860</td>
</tr>
<tr>
<td></td>
<td>199.5</td>
<td>6.31</td>
<td>4160</td>
</tr>
<tr>
<td></td>
<td>222.0</td>
<td>11.09</td>
<td>7320</td>
</tr>
<tr>
<td>2</td>
<td>187.5</td>
<td>6.69</td>
<td>4420</td>
</tr>
<tr>
<td></td>
<td>201.0</td>
<td>–3.68</td>
<td>–2430</td>
</tr>
<tr>
<td></td>
<td>212.0sh</td>
<td>–2.06</td>
<td>–1360</td>
</tr>
<tr>
<td></td>
<td>227.0</td>
<td>4.15</td>
<td>2740</td>
</tr>
<tr>
<td>3</td>
<td>190.5</td>
<td>13.60</td>
<td>193.0</td>
</tr>
<tr>
<td></td>
<td>223.5</td>
<td>7.26</td>
<td>220.5</td>
</tr>
<tr>
<td>4</td>
<td>188.0</td>
<td>10.07</td>
<td>190.0</td>
</tr>
<tr>
<td></td>
<td>198.5</td>
<td>–3.63</td>
<td>199.5</td>
</tr>
<tr>
<td></td>
<td>212.5sh</td>
<td>–0.87</td>
<td>212.5sh</td>
</tr>
<tr>
<td>5</td>
<td>222.0</td>
<td>3.10</td>
<td>227.0</td>
</tr>
<tr>
<td></td>
<td>199.5</td>
<td>13.60</td>
<td>193.0</td>
</tr>
<tr>
<td></td>
<td>219.0</td>
<td>13.05</td>
<td>218.5</td>
</tr>
</tbody>
</table>
The CD spectra of Amadori derivatives 3 and 4 in TFE resemble those of their parent peptides 1 and 2. In the spectrum of 3 the positive band of 1 at 199.5 nm is missing, the spectrum between =200 and =220 nm is positive but unstructured (Figure 2A). The solvent dependence of the CD of 3 and 4 also differs from that of 1 and 2 (Table I).

The CD spectrum in TFE of the bicyclic Amadori derivative 5 is marked by two intense positive bands (Figure 2A). The positive band near 220 nm is contributed by the aromatic residues as in the case of the open-chain Amadori derivatives. The band positions do not change significantly, but the intensities are decreased in the solvent mixture and water. These spectral features are compatible with high population of a type II β-turn showing class C’ CD spectrum.

The CD spectral parameters of imidazolidinone compounds 6–8 are summarized in Table II. The CD curves in TFE generally show not too much similarity. The only exception is cis isomer of the «free hands» compound 6b and bicyclic one 8b the CD spectra of which, with minor alterations, feature bands with the same sign pattern and intensity (Figure 2B). This suggests the foldedness of the open «free hands» compound 6b. The corresponding trans isomers 6a and 8a have very different CD spectra in TFE (Figure 2B). The CD spectra of the trans «free hands» compounds 6a (acid) and 7 (amide) are also different (Table II and Figure 2B). The spectrum of 6a shows a broad positive band with fine structure between =190 and =210 nm. All the other imidazolidinone derivatives show negative CD in this region regardless of the trans- or cis relative orientation of the aromatic chromophore and the sugar antennae. This likely reflects difference of the foldedness of the overall structure. Because of the presence of the macroring 8a and 8b are necessarily folded. As shown in Figure 2B, the cis-isomers 6b and 8b have strong positive bands at 193.5 nm and near 220 nm. This may be due to the same cis steric orientation of the aromatic chromophore and sugar antennae. Apparently, the CD spectra of imidazolidinones 6–8 are rather complicated: they are determined by the foldedness of the peptide backbone, the environment of the aromatic chromophores and the CD contribution of the imidazolidinone.

Further, it is noteworthy to observe that in the far-UV region the long-wavelength band has opposite sign in trans and cis imidazolidinone diastereoisomers (Figure 2B). The sign of this band appears to be dependent on the relative orientation of the aromatic chromophore and sugar antennae but its position and intensity are strongly affected by the polarity of the solvent. In the spectra of the cis isomers (6b and 8b) in TFE the positive bands are present only as shoulders.
As reported earlier for Amadori compound 3,22 the attachment of free sugar influences the side chain conformer distribution of Tyr and likely Phe. In agreement with this, CD spectra of 3–5 in the 1Lb region change sign relative to the band of parent Leu-enkephalins 1 and 2. The most intense vibrational fine structure is observed in the spectrum of the cyclic Amadori derivative 5 (Figure 3A).

The imidazolidinone moiety incorporated into the structure of compounds 6–8 does not influence the positive sign of the broad 1Lb band relative to parent peptides 1 and 2, but have higher intensity in the spectra of »free hands« derivatives 6a, 6b and 7 regardless of the relative orientation (trans or cis) of the ring substituents (Figures 3A and 3B).

### FTIR Spectroscopic Characterization of Compounds 1–8

The infrared spectra of Leu-enkephalin (1), Leu-enkephalin amide (2), their Amadori (3–5) and imidazolidinone derivatives (6–8) were taken in TFE in the 1800–1500 cm⁻¹ spectral region, which is mainly composed of bands due to νCD (COOH), amide I, amide II, and νas (COO⁻) vibrations (Figure 4; spectra of compounds 2 and 6b not shown). Absorptions by the aromatic Tyr and Phe side chains also give contribution in this spectral region. Amadori compounds 4 and 5 are present as trifluoroacetate (TFA⁻) salts. The strong νas (COO⁻) band of TFA⁻ appears at ~1675 cm⁻¹. Band assignment is based on the data in Scheme 1.22,26 The position of the weak bands

---

### Table II. Molar CD (Δε/deg⁻¹cm⁻¹) of compounds 6–8 in TFE, TFE-water (1:1) mixture and water

<table>
<thead>
<tr>
<th>Compound</th>
<th>TFE</th>
<th>TFE/H2O = 1/1</th>
<th>H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ / nm</td>
<td>Δε / deg⁻¹cm⁻¹</td>
<td>λ / nm</td>
</tr>
<tr>
<td>6a</td>
<td>195.5</td>
<td>3.84</td>
<td>194.5</td>
</tr>
<tr>
<td></td>
<td>200.5</td>
<td>5.26</td>
<td>204.0</td>
</tr>
<tr>
<td></td>
<td>210.0</td>
<td>3.92</td>
<td>209.0</td>
</tr>
<tr>
<td></td>
<td>217.0</td>
<td>1.98</td>
<td>215.5</td>
</tr>
<tr>
<td></td>
<td>229.0</td>
<td>-2.06</td>
<td>229.5</td>
</tr>
<tr>
<td>6b</td>
<td>193.5</td>
<td>6.37</td>
<td>194.5</td>
</tr>
<tr>
<td></td>
<td>205.5</td>
<td>-3.32</td>
<td>201.5</td>
</tr>
<tr>
<td></td>
<td>219.5</td>
<td>4.10</td>
<td>217.0</td>
</tr>
<tr>
<td>7</td>
<td>191.0</td>
<td>0.38</td>
<td>191.0</td>
</tr>
<tr>
<td></td>
<td>197.0</td>
<td>-4.12</td>
<td>198.0</td>
</tr>
<tr>
<td></td>
<td>224.0</td>
<td>-4.68</td>
<td>228.0</td>
</tr>
<tr>
<td>8a</td>
<td>193.5</td>
<td>0</td>
<td>202.0</td>
</tr>
<tr>
<td></td>
<td>214.0</td>
<td>0</td>
<td>214.0</td>
</tr>
<tr>
<td></td>
<td>227.5</td>
<td>-4.25</td>
<td>225.5</td>
</tr>
<tr>
<td>8b</td>
<td>193.5</td>
<td>6.14</td>
<td>194.0</td>
</tr>
<tr>
<td></td>
<td>203.0</td>
<td>-5.08</td>
<td>202.0</td>
</tr>
<tr>
<td></td>
<td>218.0</td>
<td>5.49</td>
<td>218.5</td>
</tr>
</tbody>
</table>

---

Figure 3. The near-UV region circular dichroism spectra in TFE of compounds 1–5 (A) and 6–8 (B). The spectra were obtained at room temperature and expressed as molar CD (Δε/deg⁻¹cm⁻¹) from 250 to 295 nm.
and shoulders have been confirmed or corrected by Fourier self-deconvolution (FSD).

The FTIR spectra of Leu-enkephalin (1) and its Amadori derivative (3) have been reported earlier.22 The data measured herein are in agreement with the published ones. The sharp component bands near $\sim 1519$, $\sim 1600$ and $\sim 1616$ cm$^{-1}$ in the infrared spectra of all analyzed compounds are assigned to the ring vibration of Tyr and Phe (see Scheme 1). Concerning the ionization state of compounds 1–8, it was observed that spectra show differences due to the $\text{NH}_2$-peptide-COOH $\rightleftharpoons$ $\text{NH}_3^+$-peptide-COO$^-$ equilibrium in TFE. In the case of compounds 1 and 3 the rather low intensity of $\nu_{\text{CO}}$ COOH and the increase and broadening of the band below $\sim 1600$ cm$^{-1}$ are an indication of the predominance of increased population of the zwitterionic form with COO$^-$. Furthermore, it appears that imidazolidinones 6a, 6b possess higher amount of the protonated species. The carboxylic $\nu_{\text{CO}}$ stretching vibration at $\sim 1720$ cm$^{-1}$ increases in both diastereoisomers while the carboxylate antisymmetric stretching band below $\sim 1600$ cm$^{-1}$ vanishes. As expected, no contributions have been observed in these two diagnostic domains in the FTIR spectra of amide compounds 2, 4 and 7. The macro-ring of the bicyclic Amadori compound 5 as well as of diastereoisomeric bicyclic imidazolidinones 8a, 8b is closed by an ester bond. In their spectra, the corresponding $\nu_{\text{CO}}$ ester band with medium intensity turns up around 1730 cm$^{-1}$. A slightly lower frequency observed for the latter two compounds may be due to the formation of H-bonds between the carbonyl oxygen and the neighboring hydroxyl groups.

As known from previous FTIR studies, Leu-enkephalin exhibits in TFE an ensemble of conformations, the main components of the amide I band were assigned to $\beta$-sheet, $\gamma$-turn, semi-open and open conformers.22 The shape and deconvolution of the amide I component bands of compounds 2–8 gave us some information about their backbone conformation. In general, the FTIR spectra of the zwitterionic compounds 1, 3 and 6a reflect in TFE solution the presence of more folded conformers ($\beta$- and/or $\gamma$-turns) than their corresponding amide analogs 2, 4 and 7. In the case of cyclic Amadori compound 5 the $\nu_{\text{as}}$ stretching band of trifluoroacetate (TFA$^-$) at $\sim 1672$ cm$^{-1}$ is overlapped with the amide I band and dominates the spectrum. However, shoulders in its FSD spectrum at 1646, 1638 and 1629 cm$^{-1}$ are present, which indicate the presence of turns. These findings are in agreement with the results of CD spectroscopy measurements. Evidence for the presence of the acyclic form of reducing sugars in solution has recently been proved by FTIR spectroscopy, the $\nu_{\text{CO}}$ band of the open-keto-chain as well as enediol absorption being found near $\sim 1728$ cm$^{-1}$ and $\sim 1647$ cm$^{-1}$, respectively.27,28 In the case of Amadori compounds 3 and 5 these spectral regions are obscured by $\nu_{\text{CO}}$ bands of COOH or ester group, respectively, and by the weak hydrogen bonds in the semi-open turns in the low-wavenumber amide I region (see Scheme 1). Therefore we can not reliably estimate the contribution of the open sugar ring. No absorption band has been observed in the $\sim$open-keto$\sim$ $\nu_{\text{CO}}$ spectral domain of Amadori compound 4. We presume that the ring oxygen protonation as promoter step for carbohydrate ring-opening...
Figure 4. Experimental FTIR spectra of compounds 1, 3–5, 6a, 7, 8a and 8b (solid line) and their Fourier self-deconvolution (FSD) traces (dashed line) assuming Lorentzian band shape and values of 30 cm\(^{-1}\) and 0.2 for the bandwidth and noise reduction parameters, respectively.
reaction is suppressed in compound 4 due to the absence of the COOH group.

The »free-hands« type imidazolidinone compounds 6a, 6b and 7 comprise a heterocyclic unit as well as sugar and peptide chains. The open sugar chain does not influence the spectrum between 1500 and 1800 cm⁻¹ but the N-substituted lactam subunite of all imidazolidinone compounds (6–8) has a characteristic spectral contribution below 1700 cm⁻¹ (Figure 4).

The main spectral difference in the FTIR spectra of the trans and cis »free-hands« imidazolidinones 6a and 6b is the increased relative intensity of the shoulder at =1690 cm⁻¹ in the spectrum of the cis form. Furthermore, the trans and cis isomers of the ester-bonded bicyclic imidazolidinone compounds 8a and 8b show a great difference in the intensity and the position of the band assigned to the imidazolidinone cis lactam bond. In the spectrum of the cis form (8b) there is a separated band at =1690 cm⁻¹ (appearing at 1693 cm⁻¹ in the FSD trace), while the corresponding band of the trans form (8a) appears only as a shoulder near 1696 cm⁻¹. This observation may be a sign of the presence of more folded conformers in the less crowded trans isomers in which, due to formation of turn(s), the νC=O–HNR band of the intramolecularly hydrogen bonded exocyclic carbonyl group is shifted to the lower spectral region.

**DISCUSSION**

The majority of small linear peptides of biological importance, like Leu-enkephalin, in aqueous solution are present as mixtures of rapidly interconverting conformers. Traditionally, CD experiments in organic solvents such as halogenated alcohols are used for exploring their tendency to adopt a detectable or even predominant amount of intra-H-bonded folded structures.29 Besides, halogenated alcohols have been suggested to mimic the microenvironment established by apolar proteins or membranes.30

In general, the CD and FTIR spectra of the zwitterionic peptide Leu-enkephalin 1, Amadori derivative 3 and trans-isomer of the »free hands« imidazolidinone 6a reflect in TFE solution more expressed foldedness than their corresponding amide analogs 2, 4 and 7, respectively. According to the literature,26 based on information obtained by CD, FTIR and/or NMR spectroscopy, there are no general rules on which glycosylation influences structural propensities of parent peptides, so it can alter backbone conformation to different degrees. Based on the far-UV CD spectra (190–250 nm), the keto-sugar attached to the N-terminus of enkephalins in compounds 3 and 4 does not have a significant effect upon the backbone conformation equilibrium of the parent peptides. While in the far-UV range information related to the secondary structure of the peptide backbone can be obtained, in the near-UV spectral region, between 250 and 300 nm, the CD of most peptides/proteins is dominated by the contributions of the aromatic amino acids Phe, Tyr and Trp, and by the disulfide chromophore of cystine.31,32 Tyr has lower symmetry than Phe and therefore has more intense absorption band in the 270–290 nm UV-range. The opposite, negative sign of the 1Lₐ band in the CD spectrum of the Amadori compounds can be explained by the difference in the orientation of the Tyr side chain. 1Lₐ transition has been found to be extremely sensitive in both sign and magnitude to rotation about the C⁵⁻O⁷ bond.33

It is an intriguing question whether or not the imidazolidinone ring, acting as a bridge between the carbohydrate and peptide antennas in the studied compounds 6–8, has a significant contribution to their conformer distribution. Correlation between the conformational geometry and the sign of Cotton effect (CE) of simple α-amino acid 4-imidazolidinones and 5-oxazolidinones, respectively, have been studied earlier on the basis of their CD spectra.25,34 It was concluded that the sign of the n→π⁺* lactam band depends on the envelope conformation of this five-membered heterocyclic ring and the position and type of the attached bulky substituents. Because of the complexity of compounds 6–8 and overlapping of n→π⁺* transition bands with the 1Lₐ bands of Tyr and Phe side-chains, it is hard to draw a clear-cut conclusion. However, since in the long-wavelength far-UV region of imidazolidinones CD spectra, all trans derivatives possess negative CE while the corresponding cis isomers have positive CE, it is reasonable to presume that these findings allow for stereochemical correlation and makes possible to distinguish between trans- and cis-substituted imidazolidinones. The imidazolidinone moiety incorporated into the structure of compounds 6–8 does not change the positive sign of the broad 1Lₐ band of the parent peptides 1 and 2 in the near-UV CD spectra.

In FTIR spectra, the N-substituted lactam subunite of all imidazolidinone compounds (6–8) has a characteristic contribution below 1700 cm⁻¹. Based on literature data, angular ring strain of the 5-membered heterocyclic imidazolidinone and oxazolidinone molecules, shifts the carbonyl stretching frequency into the higher spectral region by the order of 20–30 cm⁻¹, compared to their acyclic analogs.35,36 With a decrease in the bond angle, the carbonyl stretching mode should appear at higher frequency, since it is more difficult for the carbon atom of an exocyclic carbonyl group to move into the ring during a cycle of νC=O due to the restriction of the movement of the two adjacent atoms. Besides, the molecular orbital calculations led to the conclusion that in conjugated cyclic π-electronic molecular systems increase of angular strain is accompanied by bond hybridization changes which are reflected in higher stretching force constants and frequencies.35 The cis lactam band between 1690–1700 cm⁻¹ in the spectrum of the trans and cis »free hands« and

bicyclic imidazolidinone diastereoisomers, differs in position and intensity indicating different population of folded and extended conformers.

Due to their limited ability to form secondary structures such as α-helix, β-sheet, or random coil, cyclic peptides are very good models for turn(s).37,38 The most intense band in the IR spectrum, the amide I band (carbonyl stretching coupled with in-plane NH bending and CN stretching modes, around 1695–1610 cm⁻¹), consists of a series of overlapped component bands which occur as a result of the secondary structures present in peptides. The analysis of the amide I band permits to determine the peptide secondary structures. The low wavenumber shoulder of the amide I band may be a sign of the spectral contribution of turn(s). The amide I component band of the acceptor CO of intramolecular H-bond(s) of β-turns [1→4 (C₁₀) H-bonding] and γ-turns [1→3 (C₆) H-bonding] was suggested to appear near 1640 and 1620 cm⁻¹, respectively.24 Accordingly, FTIR spectroscopy represent an effective tool for studying the influence of the sugar residue, a rigid 5-membered imidazolidinone ring and cyclization on the structural and conformational features of parent unmodified peptides. The combined application of these two techniques allowed for detection of turns in small and midsize peptides. Moreover, the CD/FTIR approach was already applied for detection of turns in glycosylated midsize peptides. Moreover, the CD/FTIR approach was already applied for detection of turns in glycosylated midsize peptides. Moreover, the CD/FTIR approach was already applied for detection of turns in glycosylated midsize peptides.

It is shown in this paper that CD and FTIR spectroscopy represent an effective tool for studying the influence of the sugar residue, a rigid 5-membered imidazolidinone ring and cyclization on the structural and conformational features of parent unmodified peptides. The combined application of these two techniques allowed to evaluate the relative amount of the extended and folded conformers of Amadori compounds 3–5 as well as imidazolidinones 6–8.

Acknowledgements. – This work was supported by grant from the Hungarian Government (Scholarship to M.R. established on the basis of bilateral Hungarian/Croatian agreement), the Hungarian Scientific Research Fund OTKA (grants T 047186 to E.V. and NI 68466 to M.H.), the Economic Competitiveness Operational Program GVOP-KMA (GVOP-3.2.1-2004-04-0345/3.0), the Ministry of Science, Education and Sports of Croatia grant No. 098-0982933-2936, and by financial support from the bilateral Hungarian/Croatian project No. CRO-5/2006.

REFERENCES

2. J. Hughes, Brain Res. 88 (1975) 295–308.

SAŽETAK

CD i FTIR spektrokspska istraživanja ugljikohidratnih derivata opioidnih peptida

Elemér Vass, Miklós Hollósi, Štefica Horvat i Maja Roščić

Važnost i raznolikost funkcija glikoproteina u mnogim fiziološkim i patološkim procesima potakla nas je na dizajn novih tipova glikopeptidnih molekula i na istraživanje utjecaja glikacije na konformacijska obilježja ishodnih peptida. U ovom su radu prikazani rezultati CD i FTIR spektroskopskih istraživanja strukturno različitih linearnih i cikličkih glikopeptida, Amadorijevih i imidazolidinonskih spojeva 3–8, te uspoređeni sa spektroskopskim svojstvima ishodnih peptida, Leu-enkefalina (H-Tyr-Gly-Gly-Phe-Leu-OH, 1) i Leu-enkefalalin amida (2). CD i FTIR mjerenja provedena su u različitim otapalima u nastojanju da se utvrde utjecaji keto-šećera, rigidnog 5-članog imidazolidinonskog prstena i/ili ciklizacije na strukturna i konformacijska obilježja peptida. Primjenom CD i FTIR spektroskopije uspjeli smo odrediti relativnu zastupljenost istegnutih i savijenih konformerama Amadorijevih i imidazolidinonskih spojeva i dokazali da uporaba ovih tehnika predstavlja dobar alat za strukturnu karakterizaciju i dublje razumijevanje njihovih konformacijskih obilježja.