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QUARTERLY

CD investigations on conformation of H-X-(Pro)$_n$-Y-OH peptides (X = Trp, Tyr; Y = Tyr, Met); models for intramolecular long range electron transfer

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Conformations of three series of peptides: H-Trp-(Pro)$_n$-Tyr-OH (n = 1–5), H-Trp-(Pro)$_n$-Met-OH (n = 1–3) and H-Tyr-(Pro)$_n$-Met-OH (n = 1–3), used as models in studies on long range electron transfer through protein matrix, were investigated by CD spectroscopy in aqueous solution at pH 5.2 in the temperature range of 10°C–90°C. CD spectra of their component N- and C-terminal dipeptide and oligoproline fragments were also measured under similar conditions. In interpretation of the spectra the *cis* ↔ *trans* equilibrium about X-Pro bonds was taken into account and CD spectra of Trp-Pro and Tyr-Pro chromophores in *trans* and *cis* configuration of the peptide bond were evaluated. The spectra of n = 3–5 peptides from the first series and those with n = 2–3 from the other two series exhibit a strong negative band in the 202–207 nm region, the strength of which is proportional to the number of Pro residues in the (Pro)$_n$ bridge, and characterized by a large temperature decrement. In view of close similarity between characteristics of this band and the 206 nm band of aqueous oligoproline peptides (n ≥ 3), known to attain a left handed helical conformation similar to that of 3_1 helix of the all-*trans* poly-L-proline II, this band was attributed to a conformation of the latter type. H-Trp-(Pro)$_2$-Tyr-OH does not form this conformation due to sterical interaction between the two bulky aromatic side chains. Conclusions drawn from analysis of the CD spectra are supported by ^{1}H and ^{13}C NMR data reported elsewhere (Poznański *et al.*, 1993, *Biopolymers* 33, 781–795).

Knowledge of conformational properties of the title peptides is required for elucidation of molecular pathways and distance dependence of the rate of intramolecular long range electron transfer (LRET) accompanying radical transformations involving side chains of the terminal amino-acid residues: Trp$^\cdot$ → Tyr$^\cdot$ [1–7], Met(S.$^\cdot$)Br → Tyr$^\cdot$ and Met(S.$^\cdot$.Br) → Trp$^\cdot$ [2, 8]. For this purpose we determined recently by ^{1}H and ^{13}C NMR methods, in conjunction with molecular mechanics and dynamics modeling, *cis-trans* isomerization equilibria at X-Pro and Pro-Pro peptide bonds and populations of $\chi_1(t,g^+,g^-)$ rotamers at the C^α-C^β bond of aromatic side chains for these peptides in aqueous solution [9, 10]. The data obtained indicated also that (Pro)$_n$ fragments of the peptides may attain a left-handed helical conformation, similar to that of the all-*trans* poly-L-proline (PLP II) [11]. It is quite commonly accepted [12–20] that a strong negative band at about 206 nm in the spectra of oligoproline peptides, the strength of which tends to approach that of the corresponding band in the spectrum of PLP II with

the growing number of Pro residues and depends characteristically on temperature, can be regarded as manifestation of attainment by these peptides of a PLP II-like conformation. In this work, we applied thus the circular dichroism approach to evaluate the extent and relative thermal stability of a similar conformation assumed by $(Pro)_n$ tracts in the three groups of the peptides studied: H-Trp-$(Pro)_n$-Tyr-OH (n = 1–5), H-Trp-$(Pro)_n$-Met-OH and H-Tyr-$(Pro)_n$-Met-OH (n = 1–3). For elucidation of the origin of the CD spectral pattern observed, CD spectra of particular building fragments of the peptides were also determined under the same conditions. The CD data obtained are discussed in connection with conformational preferences of the peptide side chains, deduced from our NMR and molecular modeling studies [9, 10].

MATERIALS AND METHODS

Peptides. The peptides: H-Trp-$(Pro)_n$-Tyr-OH, n = 1–5, H-Trp-$(Pro)_n$-Met-OH, n = 1–3, and H-Tyr-$(Pro)_n$-Met-OH, n = 1–3, and H-Trp-Pro-OCH_3, synthesized and purified by Dr. M. Ciurak (Institute of Chemistry, Gdańsk University), were of the lots used in our previous pulse radiolysis [1, 2, 6, 8] and NMR studies [9]. H-$(Pro)_n$-OH (n = 2–4), H-Trp-Pro-OH, H-Pro-Trp-OH, H-Tyr-Pro-OH, H-Pro-Tyr-OH and H-Pro-Met-OH were obtained from BACHEM and used without further purification.

CD measurements. CD spectra were recorded on an AVIV 62DS spectrometer, equipped with a termostated sample compartment, using 1 mm pathlength cells at a spectral bandwidth of 1.5 nm, a step resolution of 0.5 nm and averaging time of 1 s. Peptides were dissolved in 0.01 M Hepes buffer at pH 5.2, at which they occur for the most part in the zwitterionic form. Their concentration was $\approx 10^{-4}$ M per amino-acid residue. Before recording the peptide spectra, a blank spectrum of the buffer solution was subtracted. Molar ellipticities determined at higher temperatures were corrected for thermal expansion of the solvent. The molar concentrations of peptide solutions for peptides containing Trp and/or Tyr were determined spectrophotometrically using known molar extinction coefficients of these amino acids in the near UV region [21]. In view of the lack of reliable UV absorption data for H-$(Pro)_n$-OH peptides, $\varepsilon = 7.4 \times 10^3\ M^{-1}\ cm^{-1}$ per Pro residue at 200 nm was determined with help of ^1H NMR by measuring relative molar content of these peptides in solutions containing L-tryptophan of known molar concentration. This method was also applied for determination of molar concentration of H-Pro-Met-OH.

Trans and *cis*(X-Pro) isomers of the peptides from the three series occur in aqueous solutions in comparable amounts (cf. legends to Figs. 1 and 2), while the content of *cis*(Pro-Pro) isomers does not exceed 0.1–0.2 molar fraction [9, 10]. Therefore, in analysis of the CD spectra, contributions from the X-Pro bonds both in *cis* and *trans* configuration were evaluated as described below, but those resulting from the presence of *cis*(Pro-Pro) bonds could be neglected.

Approximate CD spectra of *cis*(Trp-Pro) and *trans*(Trp-Pro) dipeptide fragments were evaluated from the spectra of H-Trp-Pro-OH and H-Trp-Pro-OCH_3 dipeptides (Fig. 1), assuming that the latter represent a linear superposition of the spectra of their respective *cis* and *trans* isomers:

$$[\Theta](\lambda) = f_t\,[\Theta]^t(\lambda) + f_c\,[\Theta]^c(\lambda),$$

where f_t and f_c are known molar fractions of the two isomers in aqueous solution, different for each peptide [9, 10]. This assumption is justified since conformation of the Trp side chain in the respective isomers of the two dipeptides was found to be similar, and close to that observed for corresponding isomers of H-Trp-$(Pro)_n$-Tyr-OH peptides [9, 10]. The spectra of *cis* and *trans* Tyr-Pro fragments were calculated similarly from the spectra of zwitterionic and cationic forms of H-Tyr-Pro-OH dipeptide (Fig. 2), differing greatly in *cis* ↔ *trans* equilibrium but exhibiting very similar conformational preference at the χ_1(Tyr) dihedral angle within each class of isomers [10].

RESULTS AND DISCUSSION

H-Trp-$(Pro)_n$-Tyr-OH, n = 1–5, series of peptides

CD spectra of zwitterionic forms of the H-Trp-$(Pro)_n$-Tyr-OH peptides (Fig. 1) contain most of the characteristic features observed in the spectra of their building blocks, i.e. N-terminal Trp-

Pro and C-terminal Pro-Tyr dipeptides (Fig. 1), and oligoproline (Pro)$_n$ tracts [12,13].

A strong positive band at 224 nm contains contributions due to the amide n,π* transition within the component Trp-Pro, (Pro)$_n$ and Pro-Tyr fragments coupled to π,π* transitions of indole (^1B_b) and phenol (^1L_a) rings of Trp and Tyr side chains, respectively [20, 22, 23]. Coupling between these transitions is known to be strongly dependent both on the backbone and side chain conformation of the aromatic and adjacent (Pro in this case) amino-acid residues, manifesting itself in variable strength of respective CD bands [22–26]. Indeed, the ellipticity of the 224 nm band depends characteristically on n: it increases up to n = 3 and then attains a constant value. This can be taken as an indication that beginning with 3 Pro residues in the bridge each of the two terminal aromatic residues assumes a similar conformation. ^{1}H NMR data [9] lend support to this conclusion by showing that distribution of side chain χ_1 rotamers of Trp and Tyr is the same in all these peptides. Lower ellipticity of 224 nm band in the short-bridged peptides, n = 1–2, is due to steric interactions between the closely spaced aromatic side chains, which manifest themselves in restricted rotation of the Tyr side chain about the C^α-C^β bond [9].

The Trp-Pro chromophore contributes about four times as much intensity to the observed 224 nm band in *trans* as in *cis* configuration of the peptide bond (cf. respective spectra in Fig. 1, calculated as explained under Methods). The very large difference in the intensity of this band between the two spectra reflects also the somewhat different conformation of the Trp side chain in *cis* and *trans* forms of the Trp-Pro bond [9, 10]. In *cis* form, the Trp side chain is characterized by a highly preferred $\chi_1(t),\chi_2(-)$ conformation and a very close approach of (i) the H^α(Trp) and the carbonyl group of adjacent Pro_1 residue, and (ii) H^α(Pro) and the indole ring. On the other hand, in *trans* isomers rotation of Trp about C^α-C^β bond is much less restricted.

Contribution of the C-terminal *trans*(Pro-Tyr) chromophore to the 224 nm band is comparable to that of *trans*(Trp-Pro) (cf. spectra of these fragments in Fig. 1). The positive n,π* band of (Pro)$_n$ bridge is expected to contribute little intensity to CD in this region because of its very low strength [12–15].

In the spectra of longer peptides, n ≥ 3, a strong negative band is seen at 207 nm. It gains intensity with the increasing number of Pro residues (cf. Fig. 1), as it has been observed for the analogous band in the spectra of H-(Pro)$_n$-OH and H-Gly-(Pro)$_n$-OH peptides, attributed to a PLP II-like conformation [12, 15, 18]. In the CD spectrum of PLP II, similarly located band has been assigned to the longest wavelength component of the peptide π,π* exciton band [20]. These observations allow us to propose that the (Pro)$_n$ bridge in all-*trans* forms of H-Trp-(Pro)$_n$-Tyr-OH peptides, n = 3–5, adopts also a conformation similar to that of PLP II. In *cis*(Trp-Pro) isomers, this conformation is expected to be limited to (Pro)$_{n-1}$ fragment. Formation of a PLP II-like conformation by (Pro)$_n$ and (Pro)$_{n-1}$ tracts in the n = 3–5 peptides finds additional support in the results of studies on the effect of temperature (see below).

The 207 nm band is partially overlapped by strong neighbouring bands, the positive one of *trans*(Pro-Tyr) at 200 nm, and two negative ones at 210 nm and 200 nm, corresponding, respectively, to *cis* and *trans* isomers at the Trp-Pro peptide bond (cf. Fig. 1); they are ascribable [20] to the coupled π,π* transitions of a secondary amide (NV_1) and aromatic chromophores: ^1B_b of phenol and ^1B_a of indole ring. Superposition of these components in the spectra of shorter peptides (n = 1–2) gives rise to a strong positive band in the region of 199 nm. In the spectra of their longer homologues (n = 3–5), the maximum of this band is seen at negative ellipticities and its apparent intensity falls down progressively as n increases, due to overlapping by the negative 207 nm band of the (Pro)$_n$ bridge the intensity of which increases with n at a similar pace.

A fourth negative band at 188–189 nm is for the most part made of the short-wavelength n′,π* Pro-Tyr peptide and phenolic ^1B_a transitions (cf. respective spectra in Fig. 1). Its apparent intensity is modulated by the positive CD band at 194 nm of the Trp-Pro chromophore in *cis* configuration of the peptide bond (Fig. 1), and has the same origin as the negative 200 nm band of the *trans*(Trp-Pro) dipeptide fragment. In longer peptides (n = 3–5) the strength of this band is independent of the length of the (Pro)$_n$ bridge owing to similar conformation of their Trp and Tyr side chains, discussed earlier in the text.

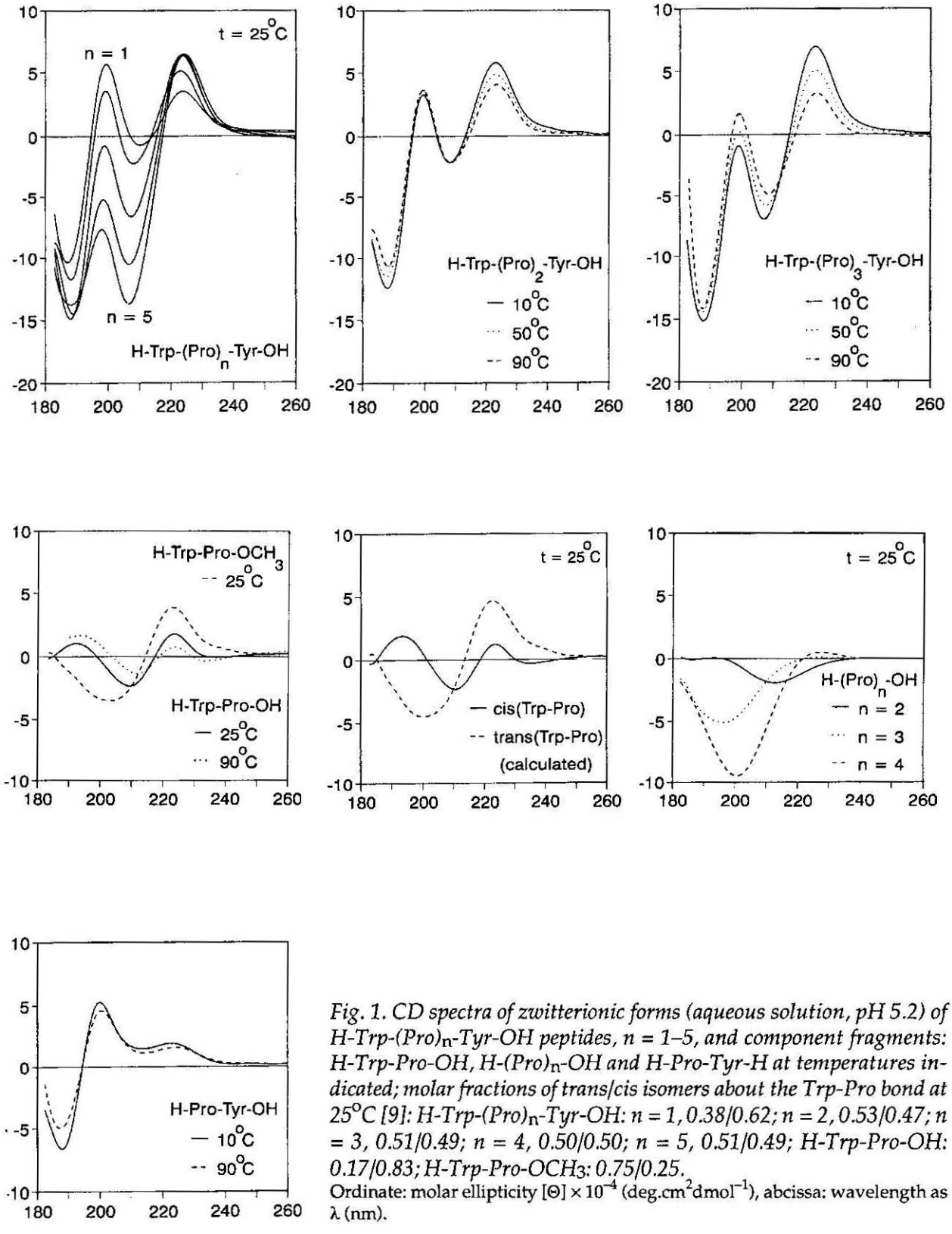

Fig. 1. CD spectra of zwitterionic forms (aqueous solution, pH 5.2) of H-Trp-(Pro)$_n$-Tyr-OH peptides, n = 1–5, and component fragments: H-Trp-Pro-OH, H-(Pro)$_n$-OH and H-Pro-Tyr-H at temperatures indicated; molar fractions of trans/cis isomers about the Trp-Pro bond at 25°C [9]: H-Trp-(Pro)$_n$-Tyr-OH: n = 1, 0.38/0.62; n = 2, 0.53/0.47; n = 3, 0.51/0.49; n = 4, 0.50/0.50; n = 5, 0.51/0.49; H-Trp-Pro-OH: 0.17/0.83; H-Trp-Pro-OCH3: 0.75/0.25.

Ordinate: molar ellipticity $[\Theta] \times 10^{-4}$ (deg.cm^{2}dmol^{-1}), abcissa: wavelength as λ (nm).

The CD spectra of longer peptides (n = 3–5) proved sensitive to temperature in a way indicative of occurrence of a thermally induced continuous conformational transition(s), characterized by a linear decrease of the strength of the 224 nm and 207 nm bands in the 10°C–90°C temperature range, leading to appearance of an isochromic point at about 211 nm (cf. Fig. 1). The strength of the positive 199 nm band falls down also with a rise in temperature. Note that the spectrum of H-Trp-(Pro)$_2$-Tyr-OH in the 200–210 nm region appeared practically insensitive to temperature (cf. Fig.1). The large value of the $\delta[\Theta]/\delta T$ decrement for the 224 nm band, of the order of 500 deg.cm^2 dmol^{-1}K^{-1}, is practically independent of the number of Pro residues in the bridge (n = 3–5) and distinctly larger than those found for this band in the spectra of H-Trp-Pro-OH and n = 1, 2 peptides (–150, –200 and –360 deg.cm^{2}dmol^{-1}K^{-1}, respectively). However, the relative decrease of the strength of this band over the whole studied temperature range of 10°C–90°C, *viz.* $\Delta[\Theta]$-(10°C–90°C)/$[\Theta]$(10°C), is of the same order of magnitude (about 0.5) for all the n = 1–5 peptides. Since the strength of the 223 nm band of the C-terminal H-Pro-Tyr-OH dipeptide proved practically temperature independent (cf. Fig. 1), the measured $\delta[\Theta]/\delta T$ decrement can be ascribed to relaxation of the specific dominant conformation of the N-terminal Trp-Pro fragment, characterized by short-range interactions between Trp and Pro$_1$ residues [9] described earlier in this paper. A distinctly smaller linear decrease in the strength of the negative 207 nm band, assigned to a PLP II-like conformation of the (Pro)$_n$ bridge, closely resembles that observed in the spectra of H-(Pro)$_n$-OH and H-Gly-(Pro)$_n$-OH peptides [12] for the corresponding PLP II-like band. Thus, we are tempted to interpret this decrease as manifestation of a gradual thermal distortion of a left-handed helical PLP II-like conformation of (Pro)$_n$ and (Pro)$_{n-1}$ bridges towards a more extended helix, as proposed recently by Dukor & Keiderling [13]. Values of the relative change in the strength of the 207 nm band over the studied temperature interval, $\Delta[\Theta]$(10°C–90°C)/$[\Theta]$(10°C), decrease with the growing number n of Pro residues (0.33, 0.20 and 0.14 for n = 3, 4, 5, respectively). This suggests that thermal stability of the helical conformation of the oligoproline fragments increases in that order. Since the corresponding values for the 224 nm band were found much larger and independent of n ≥ 3, conformation of the two peptide fragments in question does not seem to be strongly correlated.

H-Trp-(Pro)$_n$-Met-OH, n = 1–3, series of peptides

CD spectra of zwitterionic forms of H-Trp-(Pro)$_n$-Met-OH peptides (Fig. 2) are dominated by a strong positive band at 223 nm, the strength of which somewhat increases with n, and by a broad negative band at 203 nm, the intensity of which increases dramatically with the number of Pro residues beginning with the n = 1 → 2 step, and does so also at the next n = 2 → 3 step. The 223 nm band contains contributions from the Trp-Pro and (Pro)$_n$ fragments, discussed in the preceding paragraph, and from the n,π* transition within the C-terminal Pro-Met dipeptide. The latter chromophore, however, adds little intensity to the spectra in this region (Fig. 2). Distribution of χ_1(Trp) side chain rotamers in all the peptides, n = 1–3, was found very similar [10] and close to that observed in longer peptides from the H-Trp-(Pro)$_n$-Tyr-OH family [9]. This explains the weak dependence of $[\Theta]_{223}$ on length of the (Pro)$_n$ bridge. Within the 203 nm band one would expect contributions from the Trp-Pro chromophore both in *trans* and *cis* configuration of the peptide bond, proportional to the relative content of respective isomers in solution and, in the spectra of n = 2, 3 peptides, also from the (Pro)$_n$ chromophore. However, the strength of this band is much higher than it could be expected from the spectra of component chromophores and actual *cis* ↔ *trans* equilibrium about the Trp-Pro bond. It exceeds also that found for the PLP II-like band in the spectra of corresponding peptides from the H-Trp-(Pro)$_n$-Tyr-OH series, where the apparent intensity of this band is partially compensated by the positive π,π* band of Pro-Tyr chromophore. This can be in a part due to a higher backbone rigidity of (Pro)$_n$ bridge in H-Trp-(Pro)$_n$-Met-OH peptides. This point finds support in NMR data [10], according to which the *cis* ↔ *trans* equilibrium about Pro-Pro bonds in this series of peptides is completely shifted towards the *trans* form, while in the H-Trp-(Pro)$_n$-Tyr-OH series isomers with one or more Pro-Pro bonds in *cis* configuration constitute up to 0.2 molar fraction of peptides [9].

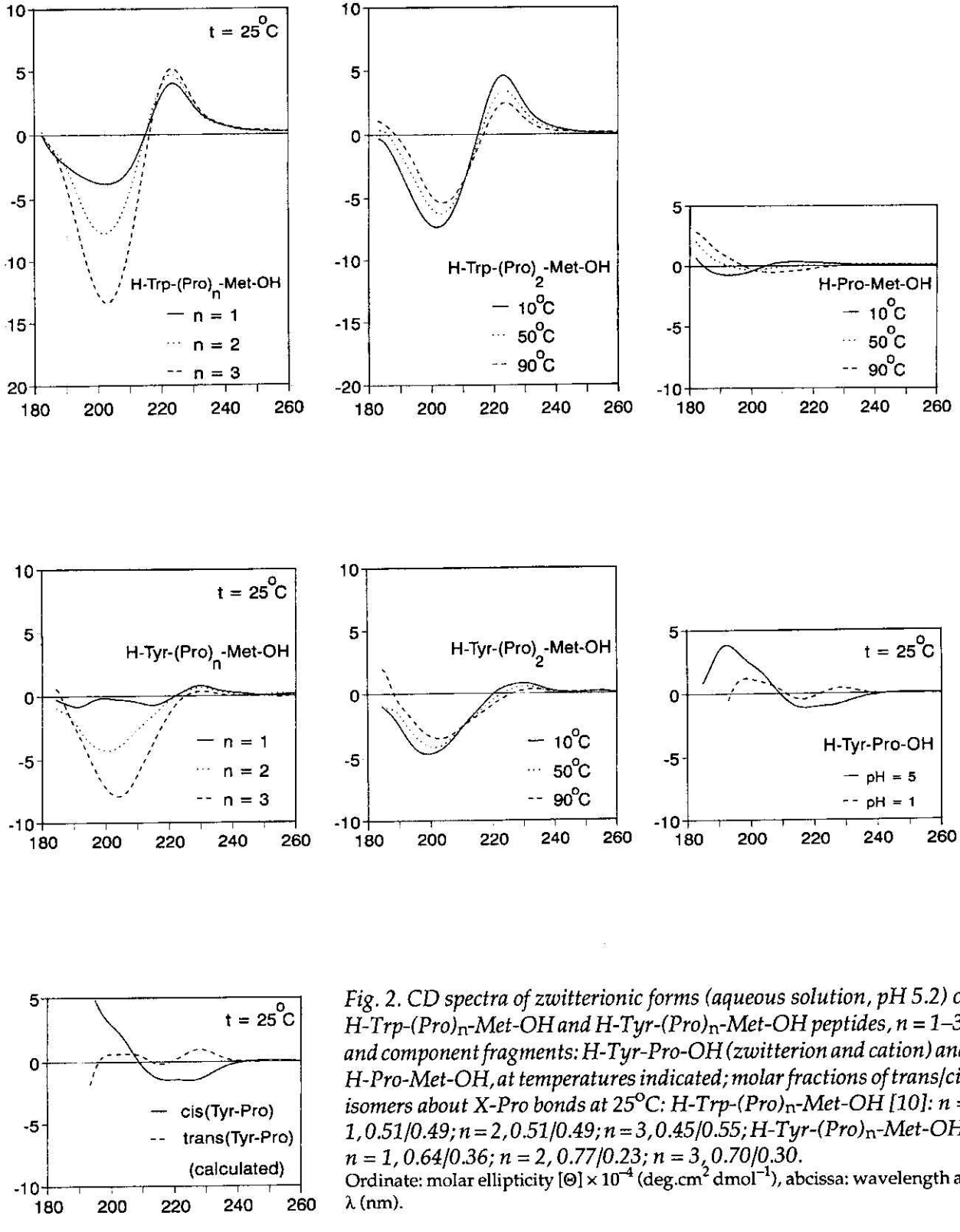

Fig. 2. CD spectra of zwitterionic forms (aqueous solution, pH 5.2) of H-Trp-(Pro)$_n$-Met-OH and H-Tyr-(Pro)$_n$-Met-OH peptides, n = 1–3, and component fragments: H-Tyr-Pro-OH (zwitterion and cation) and H-Pro-Met-OH, at temperatures indicated; molar fractions of trans/cis isomers about X-Pro bonds at 25°C: H-Trp-(Pro)$_n$-Met-OH [10]: n = 1, 0.51/0.49; n = 2, 0.51/0.49; n = 3, 0.45/0.55; H-Tyr-(Pro)$_n$-Met-OH: n = 1, 0.64/0.36; n = 2, 0.77/0.23; n = 3, 0.70/0.30.
Ordinate: molar ellipticity $[\Theta] \times 10^{-4}$ (deg.cm^2 dmol^{-1}), abcissa: wavelength as λ (nm).

Both 223 nm and 203 nm bands of n = 2, 3 peptides exhibit a linear temperature dependence leading to a decrease in their strength with formation of an isochromic point at 211 nm and 213 nm, respectively (cf. spectra of H-Trp-(Pro)$_2$-Met-OH at a number of temperatures in Fig. 2). The temperature effect is qualitatively and quantitatively very similar to that found for the H-Trp-(Pro)$_n$-Tyr-OH series: values of the coefficient $\Delta[\Theta](10^\circ C-90^\circ C)/[\Theta](10^\circ C)$ for corresponding bands of the two peptides (about 0.5 and 0.3, at 223 nm and 203 nm, respectively) are very similar to each other and to those determined for H-Trp-(Pro)$_3$-Tyr-OH.

Based on the presented analysis of CD spectra and their temperature dependence, most of the strength of the 203 nm band of either peptide, H-Trp-(Pro)$_2$-Met-OH and H-Trp-(Pro)$_3$-Met-OH, can be attributed to the longest wavelength component of the peptide π,π^* exciton transition within a helical PLP II-like conformation. A smaller part, independent of the length of the (Pro)$_n$ bridge, can be ascribed to the mixed π,π^* indole (^1B_a) and amide transition within the Trp-Pro chromophore.

The minimal requirement for nucleation of the PLP II type helical conformation in oligoproline peptides is $n \geq 3$ [12–14]. This rule is obeyed also by the H-Trp-(Pro)$_n$-Tyr-OH family, as documented in the preceding paragraph. On the contrary, this does not seem to be the case in the presently discussed group of peptides, where H-Trp-(Pro)$_2$-Met-OH exhibits the CD pattern indicative of this type backbone conformation. It seems thus that di-proline fragment is able to begin to nucleate the helical PLP II type conformation in all-*trans* isomers of H-X-(Pro)$_n$-Y-OH proline-bridged peptides provided that side chains of the N- and C-terminal amino acids do not interact strongly with one another (unlike short-bridged peptides with terminal Trp and Tyr). This point of view finds support in CD data for an analogous group of peptides, H-Tyr-(Pro)$_n$-Met-OH, presented in the next paragraph, as well as in some earlier literature data for the H-Gly-(Pro)$_n$-OH family [12] and more recent data [27] for synthetic peptides corresponding to fragments of human salivary proline-rich glycoprotein (PRG), and X-ray diffraction data for the structure of tBoc-Pro-Pro-OH in a new monoclinic crystalline form [28]. In the case of H-Gly-(Pro)$_2$-OH at low pH, the values of residual ellipticity at the positive 224 nm band and at the negative one at 197 nm, as well as the temperature decrement $\delta[\Theta]/\delta T$ for the latter band, were found of the same order of magnitude as those for its higher homologues [12]. This indicates that the latter tripeptide tends to attain a helical conformation of the PLP II type. Loomis *et al.* [27] came to the same conclusion studying pH dependence of CD spectra of a series of PRG peptides: NH_2-Gly-(Pro)$_n$-$CONH_2$, n = 2–4. The structure of tBoc-Pro-Pro-OH demonstrates [28] that the incipient formation of poly-L-proline type I and II structure requires only two consecutive proline residues [28].

H-Tyr-(Pro)$_n$-Met-OH, n = 1–3, series of peptides

CD spectra of n = 2, 3 peptides from this family (Fig. 2) are dominated by a strong negative band located at 201 nm or 204 nm, respectively, the strength of which depends on n and temperature, like in the two former series of the peptides examined. This band can be thus assigned analogously to the π,π^* peptide exciton transition within a PLP II-like conformation of the peptide backbone. Like in the case of the H-Trp-(Pro)$_n$-Met-OH series of peptides, this band appears at the $n = 1 \rightarrow n = 2$ step of the (Pro)$_n$ bridge elongation, supporting the notion that only two sequential prolines can possess the torsion angles required to nucleate the PLP II structure [27, 28]. Neither the Pro-Met nor Tyr-Pro chromophore contributes to this band significantly; the former because of its negligible optical activity while the latter mainly due to a large difference in molar ellipticity between *trans*(Tyr-Pro) and *cis*(Tyr-Pro) configurations of the peptide bond and dominance in solution ($f_t \cong 0.7$) of the more weakly absorbing *trans*(Tyr-Pro) isomer (cf. respective data in Fig. 2).

The other band seen in the spectra at about 230 nm and positive ellipticity is ascribable to n,π^* peptide transitions within Tyr-Pro, (Pro)$_n$ and Pro-Met chromophores. Its rather low strength diminishes further as n increases. Analysis of its origin indicated that it is made for the most part of contribution of the Tyr-Pro chromophore in *trans* configuration ($f_t \cong 0.7$). Note that the sign of the Cotton effect for this chromo-

phore in the 220–240 nm region is different in *trans* and *cis* configuration of the peptide bond (cf. respective spectra in Fig. 2). In the case of n,π^* transition within the Trp-Pro chromophore, the two configurations differ greatly in the strength but not in the sign of the Cotton effect.

CONCLUDING REMARKS

Analysis of the CD data obtained for the three series of oligoproline bridged peptides suggests that the peptides of $n \geq 2$ tend to assume in H_2O a helical backbone conformation as the dominant form, similar to that of all-*trans* poly-L-proline II 3_1 left-handed helix. The extent to which peptides attain this conformation depends on the configuration of the X-Pro bond, length of the $(Pro)_n$ bridge and sterical interactions between side chains of the terminal residues. When the latter do not come significantly into play, this conformation becomes nucleated in all-*trans* forms of the peptides beginning with n = 2 Pro residues in the $(Pro)_n$ bridge, as in H-Trp-$(Pro)_n$-Met-OH and H-Tyr-$(Pro)_n$-Met-OH homlogues. In the case of the H-Trp-$(Pro)_n$-Tyr-OH family, nucleation of this conformation requires n = 3 Pro residues in the bridge, owing to strong intramolecular interactions between Trp and Tyr side chains in shorter-bridged peptides. In *cis*(X-Pro) forms of all the $n \geq 3$ peptides the PLP II type conformation is limited only to the $(Pro)_{n-1}$ bridge.

Our assignment of the negative 201–207 nm bands in the CD spectra of the peptides studied to a PLP II-like conformation of the $(Pro)_n$ bridge, made on the basis of a number of analogous earlier studies for oligoproline peptides [12–14, 18, 27], should be regarded as tentative. The rather shallow potential minimum around 160° (β region of the Ramachandran plot) for conformational variation in the φ angle of *trans* peptide bond [10] suggests that in short oligoproline tracts this angle may assume a range of values corresponding to less or more tightly wound left-handed helical and β-turn conformations. Differentiation between these conformations and a one of the PLP II type is not possible solely on the basis of UV-CD spectra. However, in the light of our recent molecular dynamics simulations of conformational preferences in aqueous environment of Trp-Pro, Pro-Pro and Pro-Tyr dipeptide fragments [10], the ψ angle in the first two assumes the value characteristic for PLP II [11], while in the Pro-Tyr it undergoes fast oscillations between α (–50°) and β regions of the Ramachandran plot. These data seem to support our interpretation of the origin of 201–207 nm bands and to indicate that N-terminal Trp (Tyr) residue may be also involved in formation of a PLP-II-like structure. In this connection it is worth to note that in protein sequences X-Pro-Pro-Y there is a significant tendency for the amino-acid residues (X) flanking a Pro residue from the N-side to adopt the PLP II conformation like the proline itself [29].

The ordered helical backbone conformation of the longer peptides from the H-Trp-$(Pro)_n$-Tyr-OH ($n \geq 3$) and H-Tyr-$(Pro)_n$-Met-OH ($n \geq 2$) families proved highly relevant for interpretation of the kinetics of LRET between the radical redox pairs, Trp/Tyr and Tyr/Met.·Br, respectively, located at the terminal residues [6, 8]. We have shown [6], namely, that in short-bridged peptides (n = 0–2) of the first family electron transfer takes predominantly a through-space pathway formed by van-der-Waals contacts between properly oriented aromatic rings of Trp and Tyr. In the longer ones (n = 3–5), this transfer occurs mainly by the through-bond pathway and is characterized by a much lower value of the corresponding descriptor of exponential dependence of the rate of LRET on the distance between the redox centres than that estimated for the protein matrix. This indicates that helical segments in proteins as short as 3–5 residues may function as very efficient channels for electron transfer.

The observed thermal distortion of CD bands ascribed to a PLP II type conformation of the peptides is connected with their increased conformational dynamics and gradual expansion of a left-handed helix. This should lead to an increase in the average distance between side chains of the terminal residues and, in turn, to a decrease in the efficiency of LRET. This effect should be taken into account in interpretation of temperature dependence of the rate of electron transfer in terms of separation of nuclear

and electronic contributions to the distance dependence of the rate of LRET [6].

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