CIRCULAR DICHROISM AND CONFORMATION OF OOSTATIC PEPTIDES: THE CARRIER-LIKE ROLE OF C-TERMINAL OLIGOPROLINE SEQUENCE

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Received March 2, 2003
Accepted June 4, 2003

Circular dichroism spectra of the peptides H-Tyr-Asp-Pro-OH, H-Tyr-Asp-Pro-Ala-OH, H-Tyr-Asp-Pro-Ala-$(Pro)_n$-OH (n = 1–6) and of their two methyleneoxy isosters were measured and analyzed in terms of possible interactions between the N-terminal (mostly tetrapeptide) part that is responsible for the oostatic activity and the C-terminal oligoproline sequence. The results indicate that the two parts are largely independent and that the C-terminal $(Pro)_n$ sequence serves another purpose, possibly as a carrier.

Keywords: Peptides; Hormones; Oostatic factors; Proline; CD spectroscopy; Conformation analysis.

The oostatic factor Aed-TMOF, isolated from mosquito *Aedes aegypti* and described by Borovsky *et al.* as decapeptide H-Tyr-Asp-Pro-Ala-Pro-Pro-Pro-Pro-Pro-Pro-OH (**1**), blocks the trypsin synthesis in the midgut of blood-sucking insects and thus prevents protein digestion[1–5]. It is released from the ovary at the end of vitellogenesis and, by blocking trypsin biosynthesis, it directly precludes the oocyte growth. For several years, we have been paying attention to the decapeptide containing six cumulated proline residues at the carboxy-terminus and to its truncated analogs with shortened C-terminal sequences[6,7] (compounds **2**–**8**).

H-Tyr-Asp-Pro-Ala-$(Pro)_n$-OH

1: n = 6 **2**: n = 5 **3**: n = 4 **4**: n = 3 **5**: n = 2 **6**: n = 1
7: n = 0 (tetrapeptide, H-Tyr-Asp-Pro-Ala-OH)
8: tripeptide, H-Tyr-Asp-Pro-OH

Using these peptides we first determined their effect on insect reproduction (evolution of eggs in ovaries and hatchability of larvae of *Neobellieria bullata*, Diptera) by morphological and histological evaluation of the oostatic effect after application of the peptides by injection or after oral application[8]. We found that shortening of the C-terminal oligoproline sequence does not lead to elimination of the biological activity, and actually results in enhancement and acceleration of the oostatic effect, tetrapeptide **7** and pentapeptide **6** with either no or a very short oligoproline tail being the most active analogs. Only the shortest peptide from the investigated series, tripeptide **8**, exhibited a negligible biological activity indicating thus that the really active part of the oostatic factor **1** is its N-terminal tetrapeptide **7**. The short analogs **9** and **10** containing isosteric bond Pro-ψ[CH_2-O]-Ala have shown an even more accelerated and enhanced oostatic effect[9].

H-Tyr-Asp-Pro-ψ[CH_2-O]-Ala-X-OH

9: X = Pro **10**: X = 0

The relationship between the peptide length (*e.g.*, the number of the C-terminal Pro residues) and their oostatic effect raises the question whether there is an interaction between the N-terminal sequence and the C-terminal oligoproline part. Therefore, we attempted to evaluate the conformation of the above series of peptides with particular respect to whether there is an interaction between the two parts of the oostatic factor or whether the N- and C- terminal parts react more or less independently of the solvent and pH variation. Of various techniques of conformational investigation, the measurement and interpretation of circular dichroism data seems particularly attractive, because (i) oligoproline sequences are known to assume specific conformations (polyproline I and polyproline II), which can be recognized by CD spectra. Also, (ii) oligoproline sequences are usually rather rigid, thus making the interpretation easier. At the same time, (iii) the N-terminal tetrapeptide itself, although probably more flexible and thus vulnerable to conformational changes, contains a significant chromophore (tyrosine aromatic ring), which should be at least detectable. And also, (iv) CD spectra and conformation of oligo- and polyproline sequences have been investigated, *i.e.*, some information is available. Most recent studies of poly- and oligoproline sequences involve a combined investigation of ECD and VCD properties[10–13] including mutarotation studies of the polyproline I → polyproline II process. Conformational sensitivity of chiroptical methods brings here a distinct advantage over NMR investiga-

tion, because the latter method is somewhat handicapped in oligoproline studies due to the tertiary amide bond nature of the connecting links and the associated absence of the N–H bonds. However, numerous other studies including ORD, CD, IR and Raman spectra are available as well (see *e.g.* refs[14–16]). In this paper, we present electronic circular dichroism data of the above series of peptides together with their empirical evaluation. The forthcoming work is intended to link these data also to vibrational investigation (both the classic absorption and vibrational optical activity) and molecular dynamics simulation.

EXPERIMENTAL

Syntheses of peptides **1**–**8** and isosteric analogues **9** and **10** together with their oostatic activity bioassays have been described[6,7,9]. The model peptide **11**, H-Val-Asp-Pro-Ala-OH, has been prepared by coupling of Boc-Val-OSu with H-Asp-Pro-Ala-OH (ref.[7]) in ethanolic solution and subsequent deprotection by trifluoroacetic acid. Amino acid composition: Val (1.0), Asp (1.04), Pro (0.97), Ala (0.98). FAB MS, *m/z*: 401 ($M^+ + 1$).

Circular dichroism spectra were recorded using a computer-driven instrument, Jobin Yvon Mark VI Dichrographe. The spectra were recorded in weakly buffering aqueous media (0.01 M phosphate buffer, pH 6.5, 0.01 M NaCl/0.01 M HCl, 0.01 M NaCl/0.01 M NaOH) and in 2,2,2-trifluoroethanol (TFE) at room temperature. We used concentrations of about 1×10^{-3} mol l^{-1}, and path lengths 0.01–0.1 cm (Suprasil quartz cells). The spectra were measured as averages of 2–3 computer driven scans, each taken with the step size 0.5 nm and the instrument time constant 2 s. The spectra were baseline calibrated, further numerically evaluated and replotted as molar ellipticity per residue (deg cm^2 $dmol^{-1}$) *vs* wavelength (nm) using Spectracalc and Gramms (Galactic Industries) software packages.

RESULTS AND DISCUSSION

The complete set of CD spectra of the peptides **1**–**8** is shown in Figs 1 (neutral aqueous buffer), 2 (acidic solution, pH ≈ 2), 3 (alkaline solution, pH ≈ 12) and 4 (TFE). With a slight exception of the alkaline solution, the spectra form a very consistent picture. Starting with the hexapeptide (n = 2), positions of the two prominent CD bands (positive low-intensity band at ≈230 nm and a more intense negative band at about 195–210 nm), their absolute intensity and also the overall shapes of the CD curves resemble very closely the spectra reported for the corresponding proline oligomers in the polyproline II-type conformation. If we perform the comparison in the corresponding medium (either neutral aqueous buffer or TFE), the agreement is almost quantitative. In acidic solutions the tendency to the polyproline II-type spectrum appears even somewhat enhanced. Moreover, the curves shown for any of peptides **1** (n = 6)–**5** (n = 2) closely follow the curves reported in ref.[12] for proline oligomers with the corresponding n, *i.e.* the CD

curve of **1** follows that of $(Pro)_6$, the CD curve of **4** that of $(Pro)_3$, *etc.* This gives an indication that, at least for the longer members of the investigated series, the CD is determined by C-terminal proline residues, which adopt the polyproline II conformation and that it is the only regular peptidic structure present and thus it dominates the spectral shape. The short peptides **6**–**8** display CD spectra of different shapes; however, they again form a rather consistent set. The tripeptide **8** is characterized by a four-band CD (positive bands at 230 and 205 nm and negative bands at 215 and ≈190 nm) of rather low intensity (about one third of dichroic intensity displayed by the longer peptides). Of these bands, the two positive at 205 and 230 nm are evidently caused by the tyrosine aromatic side chain (the band

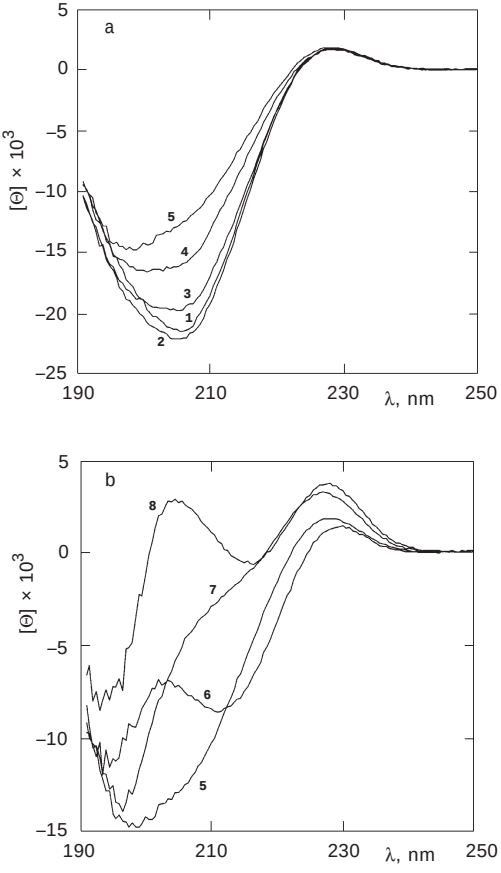

FIG. 1
CD spectra (deg cm^2 $dmol^{-1}$) of peptides **1**–**5** (a), **5**–**8** (b) in neutral buffer

at 205 nm almost completely and the band at 230 nm in part), because they clearly show a stechiometric decrease in the dichroic intensity when going towards longer peptides (there is only one tyrosine residue in any of these peptide sequences). The lowest intensity negative bands in the spectra of **8** gain more intensity with increasing number of proline residues (peptides **7** < **6** < **5** ...) and they gradually transform into a high-intensity negative band in the polyproline II-type spectra of longer peptides. The basic shape of the CD spectrum of **8** is largely independent of the solvent and solution pH and the difference between particular media consists merely in details. One tends to deduce therefore that also the N-terminal part of the

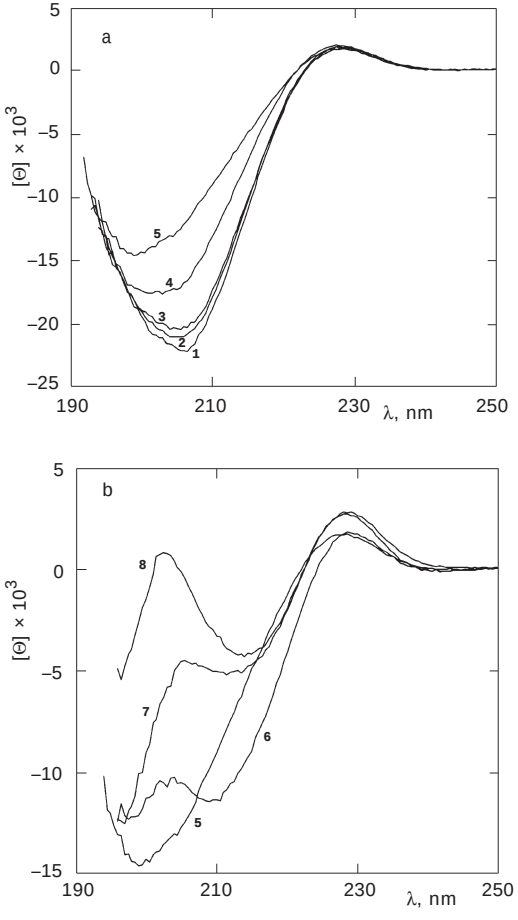

FIG. 2
CD spectra (deg cm^2 dmol^{-1}) of peptides **1**–**5** (a), **5**–**8** (b) in 0.01 M HCl

oostatic decapeptide **1** possesses a distinct, rather stable spatial structure. This, however, is in CD spectra largely masked by aromatic tyrosine bands and it is therefore not possible to infer whether it is similar to any known regular peptidic structure. The spectra measured in alkaline solutions are slightly different from the rest of the set (Fig. 3) in that the positive CD band of the polyproline II conformation (at 230 nm) is largely absent. However, the rest of the curve remains nearly the same and it is therefore quite probable that this absence is locally conditioned by ionization of the aspartic side chain and the C-terminal carboxylic group. To support further the indicated conformational independence of the two (N- and C- terminal) parts, we have attempted to construct a "theoretical" CD spectrum of tetra-

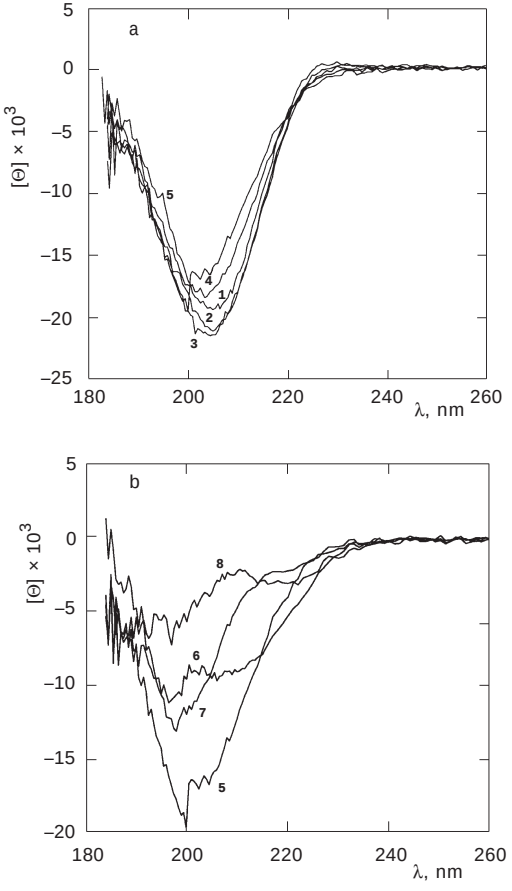

FIG. 3
CD spectra (deg cm^2 $dmol^{-1}$) of peptides **1**–**5** (a), **5**–**8** (b) in 0.01 M NaOH

peptide **7** based on the difference between the spectra of decapeptide **1** and hexapeptide **5**. This rather crude procedure is outlined as follows: (i) the spectrum of **5** is subtracted from that of **1**, giving the estimate of the CD of the four consecutive C-terminal proline residues in the polyproline II-type conformation; (ii) this difference spectrum is multiplied by 6/4 to give the estimate of the spectrum of C-terminal $(Pro)_6$ and (iii) the latter spectrum is subtracted from that of **1** giving an estimate of the CD of tetrapeptide **7**. Provided that there is no proline-induced conformational change in the N-terminal part, the two spectra should be identical. As shown in Fig. 5 (for the neutral aqueous solution), the agreement between the two spectra is quite noticeable.

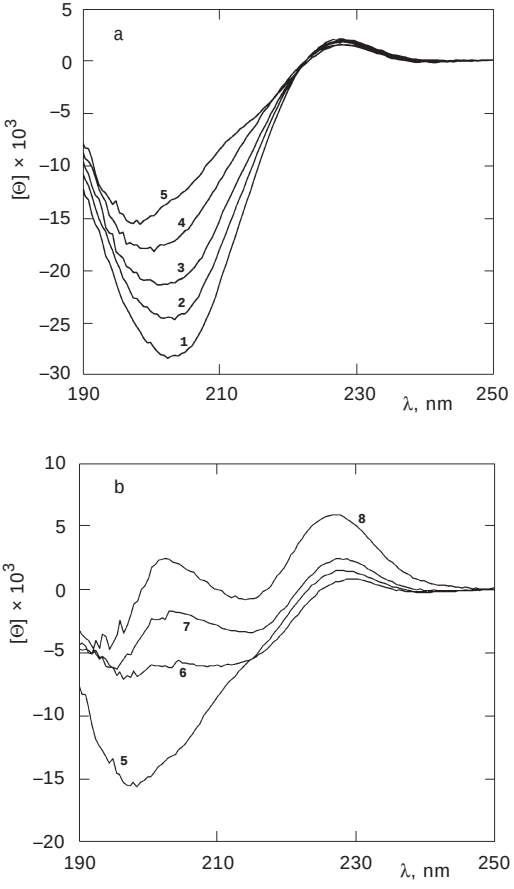

FIG. 4
CD spectra (deg cm^2 $dmol^{-1}$) of peptides **1**–**5** (a), **5**–**8** (b) in TFE

In Fig. 6, spectra of the two peptidomimetic analogs **9** and **10** are compared with those of their parent peptides **6** and **7**. They indicate the following: (i) there is an overall agreement between these spectra; thus, spatial structures od these peptides should also be quite similar in accord with the finding that the peptidomimetic analogs retain the biological activity of the non-derivatized peptides; (ii) CD of the shorter peptides is dominated

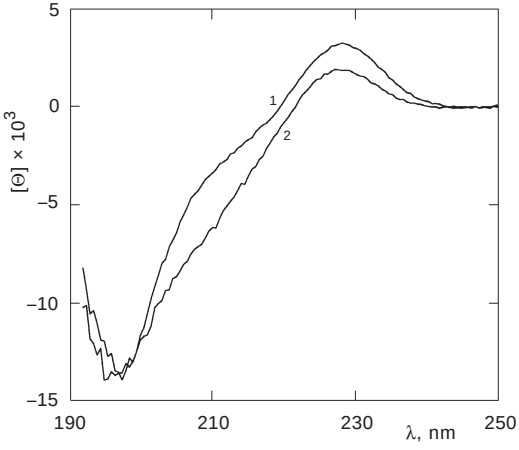

FIG. 5
Comparison of the experimental (1) CD spectrum (deg cm^2 dmol^{-1}) of the peptide **7** with its theoretical (2) model based on the hypothesis of independence of the N- and C-terminal parts

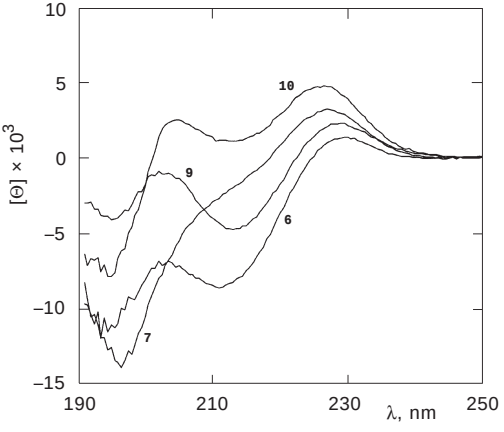

FIG. 6
Comparison of the CD spectra (deg cm^2 dmol^{-1}) of the peptidomimetic pseudopeptides **9** and **10** with their parent analogs **6** and **7**

by the tyrosine bands, because the absence of one of the peptide groups is almost unnoticeable.

In order to get more insight into conformation of the short peptides **7** and **8** we prepared the analogous peptide **11**, H-Val-Asp-Pro-Ala-OH, where the tyrosine residue in the position 1 is replaced with the sterically similar Val residue and consequently the interfering CD bands due to aromatic side chain are eliminated. CD spectra of this model peptide as shown in Fig. 7 for neutral, alkaline and acidic solution indicate again predominance of the polyproline II-type conformation despite the fact that the peptide sequence contains only one proline residue. It is therefore quite probable that such a conformation is characteristic also for the biologically active N-terminal part regardless of potential presence of the C-terminal oligoproline tail. The finding is largely not dependent of pH. When compared to the spectra of (tyrosine containing) tetrapeptide **7**, the spectra of model tetrapeptide indicate that the interfering tyrosine bands are indeed most prominent in the region between 205 and 225 nm.

In summary, CD spectra of the oostatic peptides give some support to the idea that the two parts of the molecule of **1** are indeed quite conformationally independent. The N-terminal tetrapeptide part is then probably responsible for the biological activity, while the role of the C-terminal oligoproline part is not yet known, though logically, it can serve as an inert carrier or aid in some hormonogen-like function. The C-terminal oligoproline part of these peptides assumes the polyproline II-type conformation

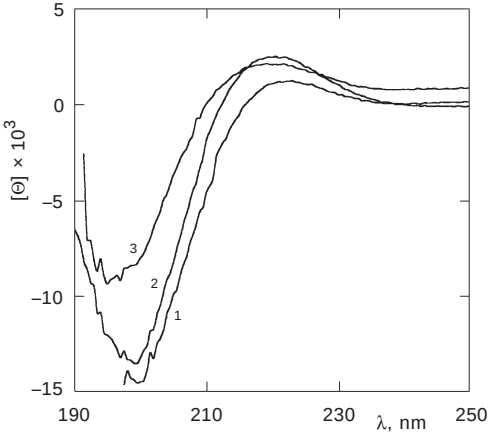

FIG. 7
CD spectra (deg cm^2 dmol^{-1}) of the model peptide **11**, H-Val-Asp-Pro-Ala-OH, in 0.01 M NaOH (1), neutral buffer (2) and 0.01 M HCl (3)

which is the most frequent spatial arrangement of oligoproline sequences in aqueous media. Further insight into conformation and time-dependent behavior of the peptides might be obtained by molecular dynamics simulations and, especially, by investigation of vibrational spectra, namely vibrational optical activity (VCD and/or ROA). The studies in this direction are in progress.

This research was carried out under the research project Z4 055 05 and was supported by the Grant Agency of the Czech Republic (grants No. 203/00/0955, No. 203/02/0247 and No. 203/02/0328).

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