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Synthesis and diuretic activities of pseudoproline-containing analogues of the insect kinin core pentapeptide

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C-2 dimethylated/unmethylated thiazolidine-4-carboxylic acid and C-2 dimethylated oxazolidine-4-carboxylic acid were introduced into the insect kinin core pentapeptide in place of Pro^3, yielding three new analogues. NMR analysis revealed that the peptide bond of Phe^2-pseudoproline (ΨPro)^3 is practically 100% in *cis* conformation in the case of dimethylated pseudoproline-containing analogues, about 50% *cis* for the thiazolidine-4-carboxylic acid analogue and about 33% *cis* for the parent Pro^3 peptide. The diuretic activities are consistent with the population of *cis* conformation of the Phe^2-ΨPro^3/Pro^3 peptide bonds, and the results confirm a *cis* Phe-Pro bond as bioactive conformation. Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

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Insect kinins share the common *C*-terminal pentapeptide sequence H-Phe-Xaa-Yaa-Trp-Gly-NH_2, where Xaa can be Tyr, His, Ser or Asn, and Yaa can be Ala but usually is Ser or Pro [1]. These kinins have been isolated from a number of insects such as *Dictyoptera, Lepidoptera* and *Orthoptera*. The first members of this insect neuropeptide family were isolated by their ability to stimulate contractions of the isolated cockroach hidgut [2], but these peptides were found to exert also potent diuretic activities that stimulate the secretion of primary urine by Malpighian tubules [3]. Structurally, the *C*-terminal pentapeptide kinin core is the minimum sequence required for full cockroach myotropic and cricket diuretic activity; therefore, it is known as the active-core region [4]. Diuretic and myotropic activities are completely lost when the *C*-terminal amide of the insect kinins is replaced by the carboxylate [5]. Within the active-core pentapeptide, position 2 tolerates wide variations in the side-chain character ranging from acidic to basic or from hydrophobic to hydrophilic [4]. Following the procedure of Fischer *et al*. [6–8] to use thioxoamide bonds in Xaa-Pro sequences to photomodulate the *cis*/*trans* conformer ratios, in previous studies we have synthesized the analogue H-Phe-TyrΨ[CS–N]Pro-Trp-Gly-NH_2 [9–11]. After UV illumination at 254 nm the *cis*-Pro conformer was found to increase from 15.7 to 47.7% in H-Phe-TyrΨ[CS–N]Pro-Trp-Gly-NH_2 with simultaneous significant (fourfold) increase in activity [9]. This structure-activity study is strongly supporting a *cis*-Pro 1–4 β-turn as the active receptor-bound conformation of the kinin pentapeptide, although a 2–5 β-turn cannot be dismissed as a candidate conformation [4,12–14].

In order to further investigate the role of *cis*/*trans* conformation of the Phe^2-Pro^3 peptide bond in diuretic activity, we designed and synthesized insect kinin core pentapeptide analogues with the Pro residue replaced by C-2 dimethylated/unmethylated pseudoprolines (ΨPro) as dimethylated pseudoprolines have previously been shown to induce in quantitative or nearly quantitative manner a *cis* conformation of the Xaa-ΨPro peptide bond [15–18]. Correspondingly, the Pro^3 residue was replaced with: C-2 dimethylated thiazolidine-4-carboxylic acid (Cys[$\Psi^{Me,Me}$Pro]) to produce H-Phe-Phe-Cys[$\Psi^{Me,Me}$Pro]-Trp-Gly-NH_2 (Thz$^{(Me,Me)}$-kinin), with C-2 dimethylated oxazolidine-4-carboxylic acid (Ser[$\Psi^{Me,Me}$Pro]) for H-Phe-Phe-Ser[$\Psi^{Me,Me}$Pro]-Trp-Gly-NH_2 (Oxa$^{(Me,Me)}$-kinin) and with thiazolidine-4-carboxylic acid (Cys[$\Psi^{H,H}$Pro]) for H-Phe-Phe-Cys[$\Psi^{H,H}$Pro]-Trp-Gly-NH_2 (Thz$^{(H,H)}$-kinin) (The structures of three analogues are shown in Supporting Information, Figures S1–S4). The parent *C*-terminal pentapeptide H-Phe-Phe-Pro-Trp-Gly-NH_2 (Pro-kinin) and Achetakinin I (H-Ser-Gly-Ala-Asp-Phe-Tyr-Pro-Trp-Gly-NH_2 [3]) were used as reference compounds.

The diuretic activities of the pseudoproline-pentapeptides were evaluated with *Periplaneta americana* cockroach hindgut myotropic assay (see Supporting Information) and the resulting dose–response curves for the parent peptide and the analogues are shown in Figure 1 and EC_{50} values are reported in Table 1. Oxa$^{(Me,Me)}$-kinin showed the highest hindguts contraction activity with a maximal response of about 120.1% of the positive control Achetakinin I. The maximal hindgut response of Pro-kinin, Thz$^{(H,H)}$-kinin and Thz$^{(Me,Me)}$-kinin is 66.8, 71.5 and 100.6%, respectively.

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Abbreviations used: *APy, (2S,4S)-4-aminopyroglutamic acid; Oxa$^{(Me,Me)}$ or Ser[$\Psi^{Me,Me}$ Pro], (S)-2,2-dimethyloxazolidine-4-carboxylic acid; Thz or Cys[$\Psi^{H,H}$ Pro], (R)-thiazolidine-4-carboxylic acid; Thz$^{(Me,Me)}$ or Cys[$\Psi^{Me,Me}$ Pro], (R)-2,2-dimethylthiazolidine-4-carboxylic acid.*

Table 1. Cockroach hindgut myotropic activities of Pro-kinin and related analogues

Peptides	Sequences	*cis* (%)	Cockroach hindgut myotropic activity		
			EC_{50} (10^{-10} M)	% Maximal response	Lasting time (s)
Pro-kinin	H-Phe-Phe-Pro-Trp-Gly-NH_2	33	7.94 ± 0.35	66.8 ± 8.2	72
$Oxa^{(Me,Me)}$-kinin	H-Phe-Phe-Ser[$\Psi^{Me,Me}$Pro]-Trp-Gly-NH_2	~100	6.14 ± 0.43	120.1 ± 12.3	120
$Thz^{(H,H)}$-kinin	H-Phe-Phe-Cys[$\Psi^{H,H}$Pro]-Trp-Gly-NH_2	50	7.36 ± 0.24	71.5 ± 14.4	78
$Thz^{(Me,Me)}$-kinin	H-Phe-Phe-Cys[$\Psi^{Me,Me}$Pro]-Trp-Gly-NH_2	~100	7.08 ± 0.39	100.6 ± 10.9	108

The maximal response is the maximal contract response of insect kinin analogue expressed as a percentage of the maximal response of Achetakinin I at 1.1×10^{-7} M concentration.

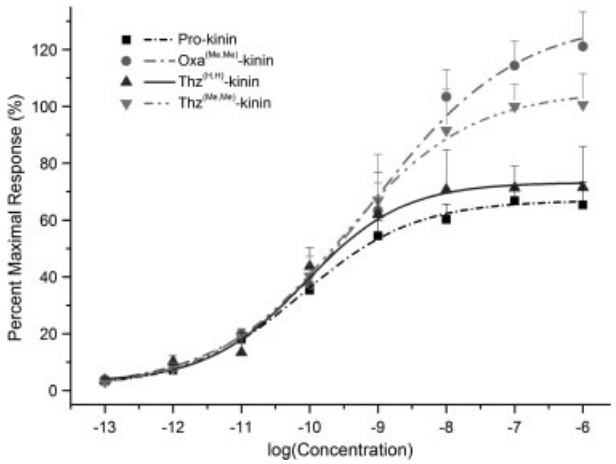

Figure 1. Comparison of the dose–response curves for the analogues and Pro-kinin. *x*-Axis represents the logarithm of molar concentration of analogues and Pro-kinin. *y*-Axis represents contraction activities expressed as a percentage of the maximal response which is a percentage of the maximal response of Achetakinin I at 1.1×10^{-7} M concentration. Data points are based on the means ±SEM of six tests. Dose–response model was used for the analysis: $y = A1 + (A2 - A1)/(1 + 10^{((LOGx0-x)\times p)})$.

The *cis/trans* ratios of the Phe-ΨPro peptide bonds were determined by integration of the Pro^3/ΨPro^3-H^{α} proton signals from the major and the minor conformation (^{1}H NMR spectra of the analogues were measured at neutral pH and shown in Supporting Information, Figures S1–S4). A doubling of resonances is clearly evident in the ^{1}H spectrum of Pro-kinin and $Thz^{(H,H)}$-kinin in D_2O, while a single set of resonances is observed for $Oxa^{(Me,Me)}$-kinin and $Thz^{(Me,Me)}$-kinin. From the integrals the *cis* contents of the Phe-Pro/ΨPro peptide bonds were calculated and the results are reported in Table 1.

The population of the *cis* isomer in the analogues is in full agreement with hindgut contraction activity. Indeed the ^{1}H NMR spectrum of the $Oxa^{(Me,Me)}$-kinin in D_2O revealed an almost exclusive *cis* conformation (~100%) of Phe^2-Ser[$\Psi^{Me,Me}$Pro]^3 peptide bond and a maximal response of up to 120.1% of Achetakinin I. Interestingly, $Thz^{(Me,Me)}$-kinin exhibits the same *cis* content as $Oxa^{(Me,Me)}$-kinin but the hindgut contraction activity is lower than that of $Oxa^{(Me,Me)}$-kinin. These different activities may well derive from steric effects as both analogues were fully soluble at 2 mM concentration in water for the NMR experiments.

In previous studies by introducing a tetrazole moiety, i.e. replacing the [-C(=O)N(H)] moiety with [-C(=N)N(N-)-] and APy residue into insect kinin the analogues H-Phe-Pheψ[CN_4]Ala-Trp-Gly-NH_2 (L,L) [19] and Ac-Arg-Phe-APy-Trp-Gly-NH_2 [14], respectively, were produced both representing *cis*-peptide bond type VI β-turn mimics. As both analogues were found to exhibit significantly enhanced activity in the cricket diuretic assay, a *cis* conformation of the Phe^2-Pro^3 peptide bond was suggested as the bioactive conformation of the insect kinin for stimulating contractions of the cockroach hidgut. Our results definitely confirm the benefits of a conformational preorganization on the bioactivities in terms of reduced entropic costs in the receptor recognition/binding process.

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Supporting information

Supporting information may be found in the online version of this article.

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