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Novel pharmaceutical composition of bradykinin potentiating penta peptide with β-cyclodextrin: Physical–chemical characterization and anti-hypertensive evaluation

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Abstract

This work describes chemical properties and anti-hypertensive activity of an oral pharmaceutical formulation obtained from the complexation of β-cyclodextrin (β-CD) with bradykinin potentiating penta peptide (BPP-5a) founded in the *Bothrops jararaca* poison. Physical chemistry characterizations were recorded in order to investigate the intermolecular interactions between species in complex. Circular dichroism data indicated conformational changes of BPP-5a upon complexation with β-CD. ROESY and theoretical calculations showed a selective approximation of triptophan moiety into cavity of β-CD. Isothermal titration calorimetry data indicated an exothermic formation of the complex, which is accomplished by reduction of entropy. The anti-hypertensive activity of the BPP-5a/β-CD complex has been evaluated in spontaneous hypertensive rats, showing better results than pure BPP-5a.

Keywords: *Bothrops jararaca*; Bradykinin potentiating peptides; ACE inhibitors; Anti-hypertensive activity; β-Cyclodextrin

1. Introduction

Toxins from snake crude venom such as *Bothrops jararaca* are very effective to kill their prey mainly by causing cardiovascular shock. The snake venom gland produces a large variety of toxins, some of them are the bradykinin potentiating peptides (BPPs) (Ferreira and Rocha e Silva, 1965). The BPPs from *B. jararaca* venom were the first identified angiotensin-I converting enzyme (ACE) inhibitors that display anti-hypertensive effect (Ferreira et al., 1970b).

The ACE is a key enzyme for the treatment of human hypertension (Ng and Vane, 1970) being responsible converting angiotensin-I into angiotensin-II, and the inactivation of vasodilator peptide bradykinin (Villard and Soubrier, 1996). The molecular features of these peptides were essential for development of the captopril, first commercial ACE inhibitor currently used for clinical cardiovascular dysfunction treatment, such as, hypertension (Ondetti et al., 1977; Cushman et al., 1979; Cushman and Ondetti, 1980).

Until now, 19 BPP sequences were found in the gland venom and some organs of *B. jararaca* (Ferreira et al., 1970a; Ondetti et al., 1971; Murayama et al., 1997; Hayashi et al., 2003; Ianzer et al., 2004). Characteristically, BPPs contain 5–14 amino acid residues with pyroglutamyl and proline residues at the N-terminal and C-terminal, respectively. Except three short peptides including BPP-5a (Pyr-Lys-Trp-Ala-Pro-OH), the others BPPs contain over 7 amino acid residues with high content of proline residues and the tripeptide Ile-Pro-Pro (IPP) at the C-

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terminal (Ianzer et al., 2004). However, the shorter BPPs, as BPP-5a, lack the tripeptide sequence IPP and are more susceptible to enzymatic hydrolysis (Ferreira et al., 1970b; Freer and Stewart, 1972) making them unable for oral administration.

On the other hand, the advances in biotechnology have accelerated the production of therapeutically active peptides and protein-based drugs used to combat a broad spectrum of diseases are in contrast with the drawbacks encountered to delivery them trough oral route, for example.

The hurdles to be overcome before oral peptides use are namely chemical and enzymatic instability, poor absorption through biological membranes, and rapid plasma clearance among others (Brayden and O'Mahony, 1998; Edery et al., 1979; Halperin et al., 1982). In addition, it is well known that drugs such as peptides and proteins could be absorbed after oral ingestion preferentially by the colon (Halperin et al., 1982; Morawietz et al., 2002). This notion stems from the assumption that the overall proteolytic activity in the colon is lower than in the stomach and small intestine. These facts in conjunction with the most accepted option by the patients, oral delivery, bring the challenge to developed new pharmaceutical formulations to overcome the drugs-peptides drawbacks.

Among the pharmaceutical formulation arsenal, the polysaccharides may play a key role to help circumvent these inconveniences (Sinha and Kumria, 2001; Rubinstein et al., 1997; Hovgaard and Brondsted, 1995; Hedges, 1998; Irie and Uekama, 1999). Polysaccharides have shown chemical resistance in other organs of digestive system. However, there is a large amount of polysaccharidases, which provokes the degradation of polysaccharides in the colon (Irie and Uekama, 1999; Sinha and Kumria, 2001; Rubinstein et al., 1997; Hovgaard and Brondsted, 1995). Cyclodextrins (CDs) are polysaccharides used in formulations, that have many advantages as peptide drug carriers candidates, in the development of advanced dosage forms, principally as excipients for oral delivery (Cramer et al., 1967; Loftsson and Brewster, 1996; Saenger, 1980; Stella et al., 1999).

CDs are cyclic oligosaccharides consisting of six to eight glucopyranose units linked by α-1,4-glycosidic bond, resulting in the formation of toroidal molecules with a polar outer surface and an apolar interior cavity. This amphiphilic characteristic makes CDs soluble and also allows the formation of supramolecular inclusion complexes with a variety of guest molecules stabilized by non-covalent interactions (Stella et al., 1999; Saenger, 1980; Cramer et al., 1967; Loftsson and Brewster, 1996).

CDs are neither hydrolyzed nor absorbed in the stomach and the small intestine. However, the vast microflora present in the colon breaks them into small saccharides, which are absorbed in the large intestine. Therefore, CDs are extensively indicated for oral administration of peptide drugs (Sinha and Kumria, 2001; Rubinstein et al., 1997; Hovgaard and Brondsted, 1995; Irie and Uekama, 1999; Hedges, 1998).

Thus, the main objective of this work was to describe a new oral pharmaceutical formulation constituted by supramolecular complex BPP-5a/β-CD. Firstly, structural characterization of pure BPP-5a was carried out by 1D (^{1}H and ^{13}C) and 2D (COSY, TOCSY, and HMQC) NMR techniques. The complex was characterized using circular dichroism, ^{1}H NMR, 2D-ROESY NMR, and isothermal titration calorimetry (ITC). In addition, theoretical studies were carried out in order to help understand the NMR experimental results. The anti-hypertensive activity of the oral pharmaceutical formulation of BPP-5a/β-CD complex was evaluated by radio-telemetry monitoring its effect on the blood pressure and heart rate of spontaneous hypertensive rats (SHR).

2. Materials and methods

2.1. Reagents

BPP-5a (Pyr-Lys-Trp-Ala-Pro-OH), P.A. degree, was purchased from Bachem®, USA Lot number 0564109 and β-CD, P.A. degree, was purchased from CERESTAR®. All reagents were used as received. During all the experiments Milli-Q® water was used.

2.2. Inclusion compound preparation

For NMR analysis and anti-hypertensive evaluation, a 1:1 BPP-5a/β-CD inclusion compound was prepared by freeze-dry method. In briefly, the BPP-5a and β-CD were dissolved in milli-Q water at 1:1 molar ratio. This mixture was submitted to stirring during 48 h. Next, the solution was freeze-dried by 48 h.

2.3. Circular dichroism

Circular dichroism spectra of BPP-5a (0.1 mM) and BPP-5a/β-CD complex (0.1 mM) solutions were recorded in duplicate with a JASCO spectrophotometer Model J-720 at 298 K in buffer KCl/HCl (pH 2), below of its isoelectric point. The BPP-5a isoelectric point was determined using the "compute pI/M_w" described at (http://us.expasy.org/tools/pi_tool.html) which is a tool that allow the computation of the theoretical pI, which gave a value of 6.10.

The wavelength was scanned from 190 to 320 nm at run time of 100 nm/min with a resolution of 0.5 nm and bandwidth of 1 nm. The spectra were the average of four scans. It was used a cell of quartz with 0.1 cm of optical length. The circular dichroism spectra were previously processed with JASCO software where were subtracted from blank. The final figures were edited with Microcal Origin 7.0.

2.4. NMR experiments

The nuclear magnetic resonance (NMR) experiments were recorded on a DPX-200 (200 MHz) and DRX-400 (400 MHz) Bruker Avance spectrometers at 300 K. BPP-5a spin systems of the individual amino acid residues were assignment through 1D and 2D NMR spectra: ^{1}H NMR; distortionless enhancement by polarization transfer, ^{13}C DEPT-135, correlation spectroscopy, COSY; total correlation spectroscopy, TOCSY (obtained at mixing time of 80, 120 and 200 ms), and heteronuclear multiple quantum coherence, HMQC. The remaining parameters were Brucker standards (Werner, 1994; Rahman, 1989).

The intermolecular interaction between β-CD and BPP-5a were monitored by ^{1}H NMR and ^{1}H–^{1}H rotating-frame nuclear overhauser spectroscopy, 2D-ROESY (500 ms spin lock) (Rahman, 1989; Werner, 1994; Schneider et al., 1998). Water suppression was achieved using the WARTERGATE technique. Data were processed using the software XWIN NMR, 3.1 (Brucker) and its edition was made with 1D Win NMR, 5.1 and 2D Win NMR 5.1.

The BPP-5a and BPP-5a/β-CD inclusion compound solutions were prepared by direct dissolution of the material in buffer KCl/HCl (pH 2) with D_2O and H_2O/D_2O (90:10, v/v) in order to obtain the NH NMR spectra. The D_2O used was obtained from Cambridge Isotope Laboratories, Inc., with 99.9% isotopic purity.

2.5. Theoretical calculation

Theoretical studies were carried out using the software packages Spartan04 (Spartan '04 Windows: Tutorial and User's Guide) and GAUSSIAN03 (Frisch et al., 2003). Geometry optimization in the vapor phase was performed using the molecular mechanics method MMFF94 (Halgren, 1996a,b,c,d, 1999, 1998; Halgren and Nachbar, 1996) and the PM3 semi-empirical method (Dewar et al., 1985). The structures obtained from theoretical calculations were characterized as true energy minima on the PES through frequency calculations (when the frequencies are real, a true minimum energy structure is present).

Conformational analyses of BPP-5a were carried out by MMFF94 and PM3 calculations with the stepwise addition of amino acid residues and geometry optimizations for fragments of BPP-5a. The addition of amino acid residues followed by geometry optimization was carried out successively until the optimization of the full peptide sequences of BPP-5a. In all cases, the calculations were performed for structures non-ionized at the two terminal ends and in gaseous phase. To investigate the interactions between BPP-5a and β-CD by theoretical methods, PM3 geometry optimizations of the BPP-5a/β-CD system were used.

2.6. Microcalorimetric measurements

Isothermal titration calorimetric (ITC) was carried out in duplicate with a VP-ITC Microcalorimeter from Microcal in buffer KCl/HCl (0.2 M, pH 2) at 298.15 K. Previously, the ITC instrument was electrically and chemically calibrated (MicroCal, 1998b; Turnbull and Daranas, 2003).

Each titration experiment consisted of 41 successive injections of BPP-5a (140 mM) into the reaction cell charged with 1.5 mL of β-CD solution (2 mM), with intervals of 350 s. The first injection of 1 μL was discarded to eliminate diffusion effects of material from syringe to cell calorimetric. After, they were injected at constant volume of 5 μL of BPP-5a. The time of injection was 2 s. The β-CD concentration in the calorimeter cell varied from 2.0 to 1.8 mM and the concentration of the BPP-5a from 0.0 to 16.5 mM. The raw data were analyzed by the software supplied with the calorimeter (Microcal Origin 5.0 for ITC), next the subtraction of blank experiment (dilution of BPP-5a in water).

2.7. Anti-hypertensive evaluation

Male SHR (14–16 weeks old) weighting 250–350 g were used. All rats were obtained from Cebio (Centro de Bioterismo do Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais). Free access was allowed to standard diet (Nuvilab CR1-Nuvital Nutrientes) and tap water was supplied *ad libitum*. The rats were housed in separated cages, under controlled conditions of temperature (298 K) and 12/12 h light/dark cycle (light: 6:00 AM–6:00 PM). Before the experiments the animals underwent acclimatization period for 12 days in an isolated telemetry room. All experimental protocols were performed in accordance with the guidelines for the human use of laboratory animals of our institution and approved by local authorities.

A telemetry system (Data Sciences International, MN) was used for measuring mean arterial pressure (MAP) and heart rate (HR). This monitoring system consists of a radio frequency transducer model TA11-PA C40, a receiver, a matrix, and an IBM-compatible personal computer with accompanying software (Dataquest A.R.T. Gold 2.0) to store and analyse the data 32. Under tribromoethanol anesthesia 2.5% (1 mL/100 g body weight), the catheter-transducer was implanted into the abdominal aorta just below the bifurcation of the iliac arteries, and the sensor was fixed to the abdominal wall. Before the experiments, the rats were housed in individual cages for 10–12 days until the telemetry tracing have indicated re-establishment of 24 h oscillations of blood pressure and heart rate. Data were sampled every 10 min for 24 h. After recovery, the SHR were randomised in three experimental groups: BPP-5a/β-CD ($n = 5$); BPP-5a ($n = 4$); vehicle β-CD ($n = 5$).

In order to characterize the cardiovascular effects of the inclusion compound in SHR, we evaluated the variation in blood pressure and heart rate and the time course and maximal effect in these cardiovascular parameters, after oral administration by gavage of the BPP-5a in a dose of 0.71 μmol/kg body weight (blank experiment) and BPP-5a/β-CD complex in a dose of 2.02 μmol/kg body weight (0.71 μmol of BPP-5a + 1.31 μmol of β-CD). The period of observation consisted of 1 h of control period followed by 10 h observation after oral administration of peptides or vehicle.

Data were colleted every 10 min during the entire experimental period. Time course: the variation in MAP was calculated each 10 min by the difference of the MAP value and an average of MAP values colleted for 1 h before administration of the compounds. Data were analyzed by two-way ANOVA followed by Bonferroni test. All statistical analyses differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Circular dichroism

Fig. 1 shows the circular dichroism spectra of BPP-5a and BPP-5a/β-CD complex. The spectrum of BPP-5a is qualitatively

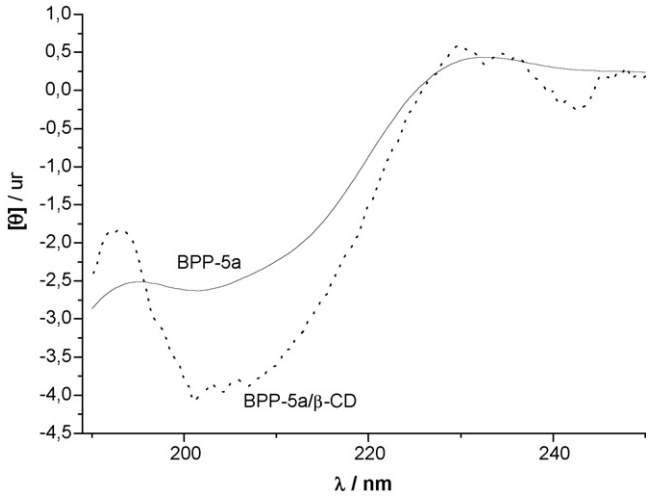


Fig. 1. Circular dichroism spectra of BPP-5a (0.1 mM; in continuum curve) and BPP-5a/β-CD complex (0.1 mM of BPP-5a and β-CD; dot curve) in buffer KCl/HCl (0.2 M, pH 2).

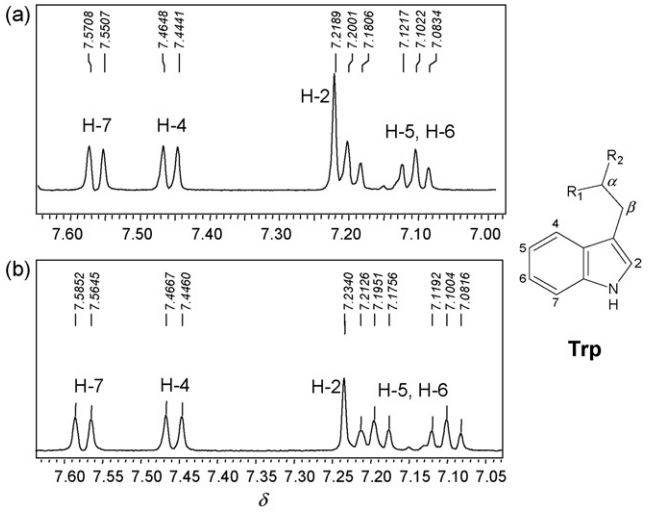


Fig. 2. Aromatic region of ^{1}H NMR (400 MHz) of: (a) BPP-5a and (b) BPP-5a/β-CD complex.

in agreement with standard data of structured small peptides (Greenfield and Fasman, 1969; Reed and Reed, 1997). BPP-5a is principally a random coil peptide, which is compatible with its short structure. Complexation with β-CD changes on the dipolar moment of BPP-5a showing consequently curve very unlike in relation to circular dichroism spectrum of BPP-5a. These results qualitatively demonstrate the host:guest interaction between BPP-5a and β-CD, suggesting greater conformational rigidity of peptide in the complex.

3.2. NMR experiments

The ^{1}H NMR spectra of the respective unbound amino acids (The Sadtler Standard Spectra, 1972) were used for help in the ^{1}H NMR assignment of BPP-5a. Normally, α-hydrogen of the peptidic bond are registered at δ_H 4.0–4.6 and β-, γ- and δ-hydrogen are registered at $\delta_H < 3.6$. The 1D (DEPT 135) and 2D (COSY, TOCSY, and HMQC) NMR experiments were also used to confirm the hydrogen and carbon chemical shifts of BPP-5a residues, as shown in Table 1.

Other investigations based on NMR analysis were carried out in order to know more information about the BPP-5a/β-CD host:guest system. The comparison between the ^{1}H NMR of the BPP-5a/D_2O, and BPP-5a/β-CD/D_2O systems was realized, as shown in Fig. 2 at aromatic region (signal attributed to tryptophan residue). The spacing of the spectral lines can be due to disturbing on the electronic density of the tryptophan aromatic ring by unbound electrons of the oxygen atoms from glycosidic unites of β-CD (Loftsson and Brewster, 1996; Schneider et al., 1998; Rekharsky et al., 1995). These differences in chemical shift are reinforced with the conformational changes observed on circular dichroism data.

Fig. 3 shows the ^{1}H–^{1}H ROESY contour map of the BPP-5a/β-CD/D_2O system. The cross-peaks on the contour map indicate proximity between nuclei within the limit of 5 Å in the space, due electromagnetic dipolar coupling (Teixeira et al., 2006; Schneider et al., 1998; Rahman, 1989; De Alvarenga et al., 2004). The correlations observed between signals of aromatic hydrogens of the tryptophan residue (δ_H 7.60–7.10) and hydrogens of β-CD (δ_H 3.90–3.70) suggest interactions between both species. Furthermore, the host:guest system is principally formed by inclusion of tryptophan residue inside the β-CD cavity.

3.3. Theoretical calculation

Geometry optimizations using theoretical calculations were carried out to obtain more information on the interaction between of BPP-5a and β-CD. The PM3 semi-empirical method was used because this level of theory has been satisfactorily employed on the studies of similar structures (Dos Santos et al., 2000). Fig. 4 depicted the minimal energy conformation of both BPP-5a, BPP-5a(I) ($\Delta H_f = -265.23$ kcal/mol), and β-CD, β-CD(I) ($\Delta H_f = -1462.51$ kcal/mol), obtained by PM3 calculations for the gaseous phase structures.

To investigate the interactions between BPP-5a and β-CD by theoretical methods, PM3 geometry optimizations of the BPP-5a/β-CD complexes constituted by BPP-5a(I) and β-CD(I)

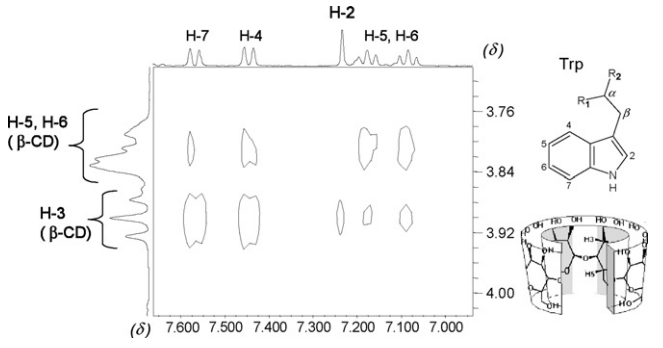
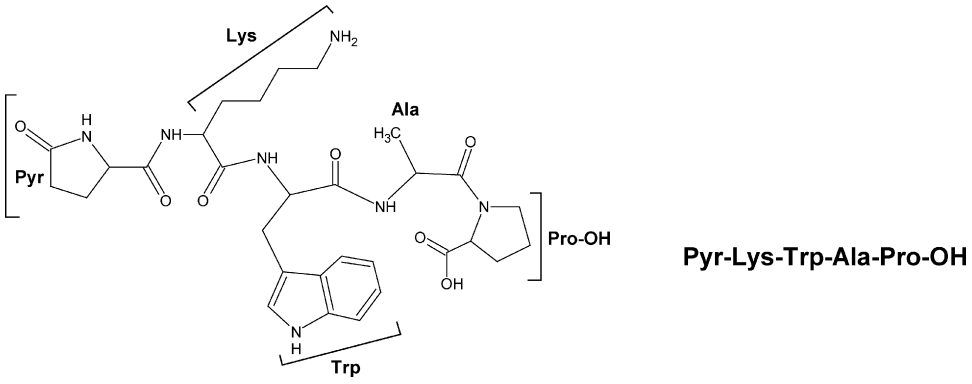


Fig. 3. Expanded ROESY (400 MHz) contour map of BPP-5a/β-CD/D_2O complex, in the region of the triptophan hydrogens (F2 from δ_H 6.8–7.6) and β-CD hydrogens (F1 from δ_H 3.3–4.0).

Table 1
Hydrogen (δ_H) and carbon (δ_C) chemical shifts on NMR spectra of BPP-5a (400 and 100 MHz, respectively) in buffer KCl/HCl (pH 2) using H_2O/D_2O (95:5, v/v) as solvent Pyr-Lys-Trp-Ala-Pro-OH



Atom	Residue									
	Pyr		Lys		Trp		Ala		Pro	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
NH	–	7.73		8.22; 7.45[a]		10.08[a]; 8.08		7.76		–
α-	53.8[b]	4.0 L[c]	59.9	4.25	54.6	4.60	47.3	4.41	56.8[b]	4.0 L[c]
β-	25.2	2.3 L[c]	25.2	2.3 L[c]	27.2	3.22	16.1	1.19	30.4	1.7 L[c]
γ-	28.3	2.3 L[c]	24.5	1.86	–	–	–	–	26.8	1.7 L[c]
δ-	–	–	29.0	2.16	–	–	–	–	39.25	2.92
ε-	–	–	47.3	3.39	–	–	–	–	–	–
2	–	–	–	–	124.6	7.29	–	–	–	–
4	–	–	–	–	111.9	7.61	–	–	–	–
5	–	–	–	–	121.9	7.2 L[c]	–	–	–	–
6	–	–	–	–	119.3	7.2 L[c]	–	–	–	–
7	–	–	–	–	118.3	7.10	–	–	–	–

[a] Attributed to amino groups of the secondary skeleton.
[b] Attribution of these signals can be changed.
[c] Large signal.

for gaseous phase structures were developed. Two types of approximations between BPP-5a(I) and β-CD(I) were considered as starting geometries for the optimizations of the BPP-5a/β-CD structure: (a) inclusion of BPP-5a(I) into the inner ring of β-CD(I) and (b) association of BPP-5a(I) with the outer face of the β-CD(I) ring. Thus, PM3 geometry optimizations were made for 40 inclusion geometries and 40 association geometries by specifying the different approximations between the amino acid residues of BPP-5a(I) and the hydroxyls of β-CD(I) in each case. The enthalpy variances of BPP-5a + β-CD → BPP-5a/β-CD process were considered as the parameter to infer about the thermodynamic favouring of the interactions in the BPP-5a/β-CD system. Thus, the interactions were considered thermodynamically favoured to geometries of the BPP-5a/β-CD complex with energies lower than $\Delta H_f = -1727.74$ kcal/mol, the value corresponding to the energy

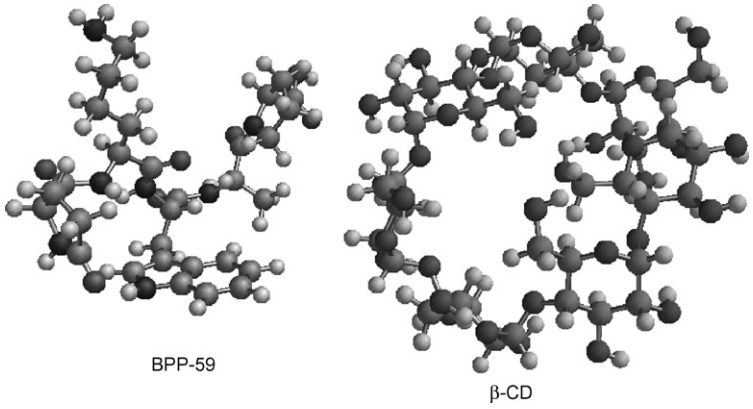


Fig. 4. Minimal energy conformations of PM3 optimized geometries of BPP-5a(I) and β-CD(I) for structures with non-ionized ends in the gaseous phase.

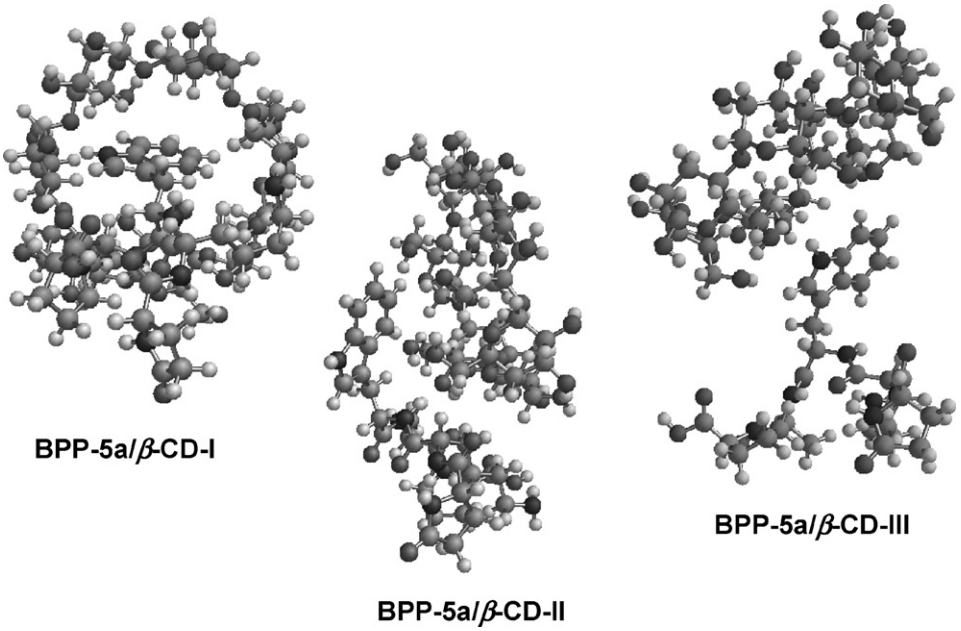


Fig. 5. PM3 optimized geometries of BPP-5a/β-CD complex: inclusion (BPP-5a/β-CD-I) and association (BPP-5a/β-CD-II and BPP-5a/β-CD-III) for structures with non-ionized ends in the gaseous phase.

sum of BPP-5a(I) ($\Delta H_f = -265.23$ kcal/mol) and β-CD(I) ($\Delta H_f = -1462.51$ kcal/mol).

Fig. 5 depicted the geometries of the BPP-5a/β-CD complexes that presented thermodynamically favored interactions, being one inclusion complex, BPP-5a/β-CD-I ($\Delta H_f = -1729.17$ kcal/mol), and two association complexes, BPP-5a/β-CD-II ($\Delta H_f = -1729.32$ kcal/mol) and BPP-5a/β-CD-III ($\Delta H_f = -1727.93$ kcal/mol). Approximations of the β-CD to BPP-5a of the tryptophan residue can be observed in these three geometries.

In the inclusion geometry, BPP-5a/β-CD-I, the shorter distances between the hydrogens of the tryptophan residue and hydrogens H-3, H-5, and H-6 of β-CD are 2.482, 2.334, and 3.231 Å, respectively. In the geometries of the association complex, the shorter distances between the hydrogens of the tryptophan ring and hydrogens H-2 and H-4 of β-CD are 3.021 and 2.514 Å, respectively, for BPP-5a/β-CD-II complex, and 2.732 and 2.986 Å, for BPP-5a/β-CD-III complex.

These theoretical results are compatible with the correlations observed in the ROESY experiment between the hydrogens at C-15 to C-19 of the guest (tryptophan moiety) and the hydrogens at C-3 and/or C-5 and C-6 of the host β-CD cavity as well as correlations between the hydrogens of tryptophan residue and the outer hydrogens of β-CD (H-2 and H-4).

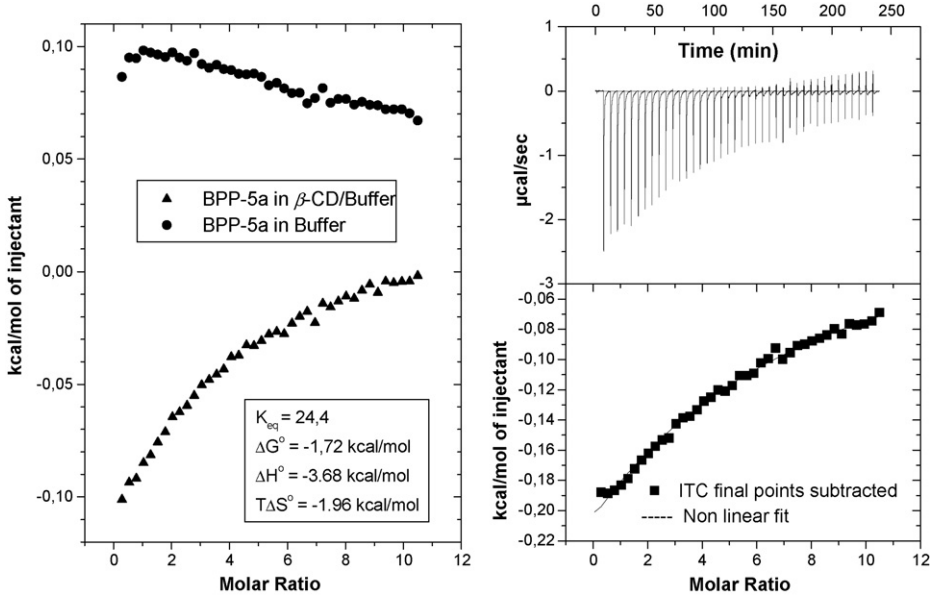


Fig. 6. ITC data for titration of BPP-5a 144 mM in β-CD 5 mM at 298.15 K, using buffer solution at pH 2.

3.4. Microcalorimetric measurements

Isothermal titration calorimetry (ITC) was used to evaluate the thermodynamic parameters of the supramolecular interaction between the species. Fig. 6 shows the BPP-5a dilution curve in buffer solution (pH 2) and the BPP-5a titration curve in β-CD in buffer solution in the same pH.

It can be observed the BPP-5a dilution is an endothermic process. This result could be due to the breakdown of the possible self-assembly peptide. In the presence of β-CD, the BPP-5a titration curve assumes exothermic values demonstrating the host:guest interaction.

The profile of ITC curve is typical of systems that exhibit weaker interactions ($K < 1000$) than highly specific ones such as enzyme-substrate ($K > 20{,}000$). Thus, there is not inflection referent to equivalence point of titration (Turnbull and Daranas, 2003; MicroCal, 1998a; Rekharsky and Inoue, 2000). However, the calculation of thermodynamic parameters was made through the non-linear fit, assuming the 1:1 stoichiometry in one set sites model present in Microcal Origin for ITC (MicroCal, 1998a).

According with thermodynamic data obtained by non-linear fit, the process is enthalpy drove ($\Delta H_i^\circ = -3.68$ kcal/mol) and

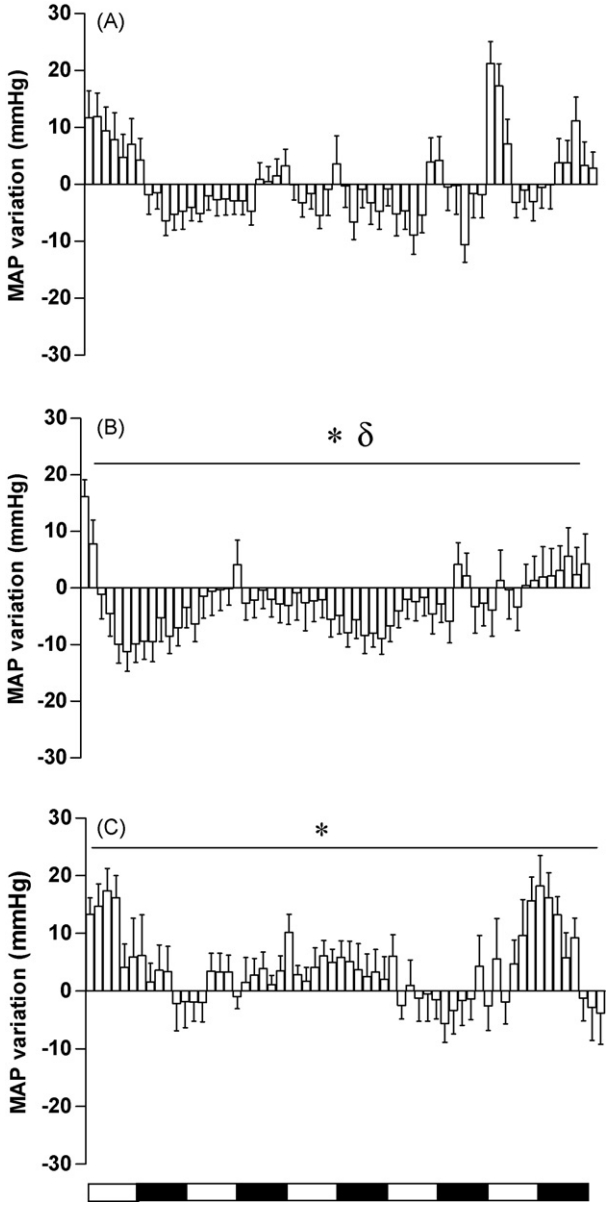


Fig. 7. Mean arterial pressure variation of SHR after BPP-5a or BPP-5a/β-CD oral administration: (A) vehicle ($n = 5$); (B) BPP-5a/β-CD ($n = 5$); (C) BPP-5a ($n = 4$). The bars show the mean variation each 10 min. Each bar at the bottom of the figure (black and white) of scale represents 1 h of record. $^{*}p = 0.010$ in comparison to vehicle; δ $p = 0.0003$ in comparison to BPP-5a (two-way ANOVA).

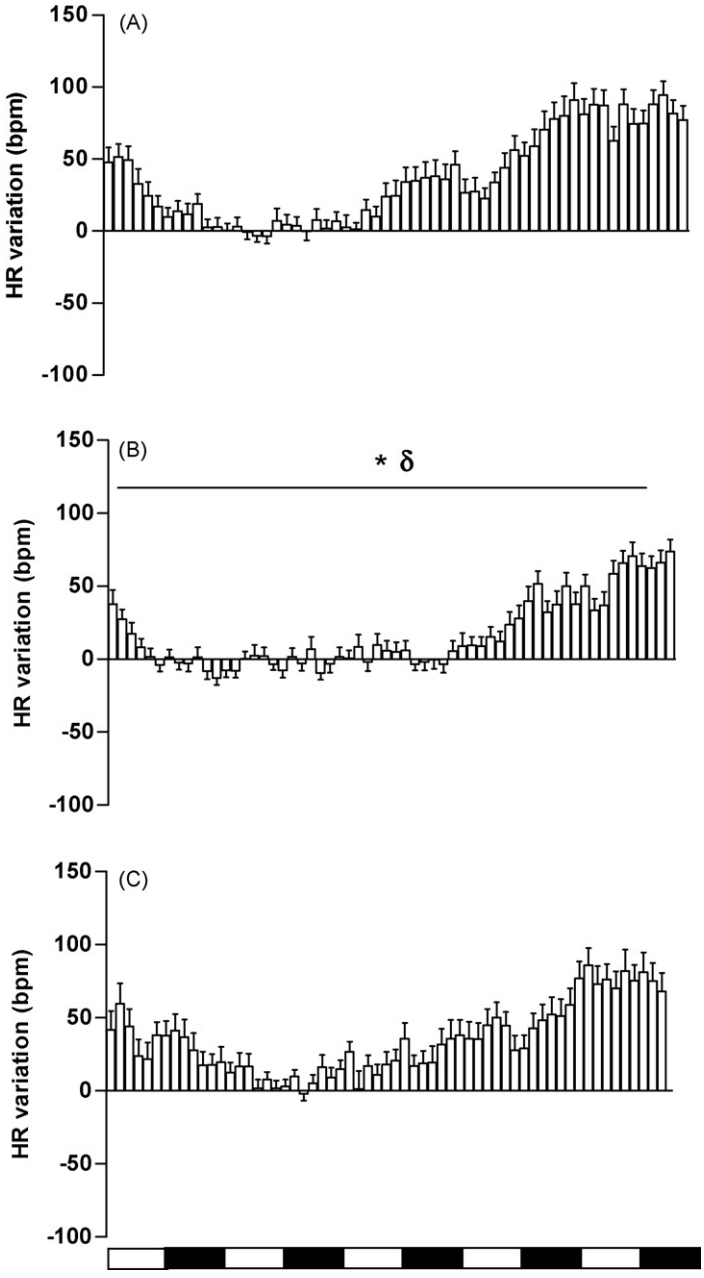


Fig. 8. Heart rate variation of SHR after BPP-5a or BPP-5a/β-CD oral administration: (A) vehicle ($n = 5$); (B) BPP-5a/β-CD complex ($n = 5$); (C) BPP-5a ($n = 4$). The bars show the mean variation each 10 min. Each bar at the bottom of the figure (black and white) of scale represents the 1 h of record. $^{*}p < 0.0001$ in comparison to vehicle; δ $p = 0.0001$ in comparison to BPP-5a (two-way ANOVA).

occurs with reduction of entropy ($T\Delta S_i^\circ = -1.96$ kcal/mol), being in accordance with literature for similar complexation. The enthalpy term was attributed to the binding of enthalpy-rich water molecules released from the β-CD cavity with bulk water molecules (Loftsson and Brewster, 1996) and the van der Waals and hydrogen bonds interactions between guest and β-CD (Rekharsky and Inoue, 1998, 2000). The entropy term could be attributed the smaller number of species on the product of reaction, greater molecular volume and different symmetry leaving to smaller rotational and translational freedom degrees of complex when compared with pure substances.

3.5. Anti-hypertensive evaluation

Figs. 7 and 8 show MAP and HR variation in SHR at 10 min interval after oral administration of BPP-5a or BPP-5a/β-CD complex. As shown in Fig. 7, the administration of 0.71 μmol/kg of BPP-5a/β-CD complex decreased MAP. The MAP decrease started with 40 min after administration and was maximal at 60 min (-11.0 ± 3.4 mmHg). The anti-hypertensive effect lasted for 6 h. A modest increase in HR, which lasted for the entire recording period, was also observed (Fig. 8). This change in HR was probably of reflex origin (baroreceptor-mediated) due to the fall in blood pressure. By contrast, oral administration of BPP-5a did not decrease MAP. Actually, a small but significant increase in MAP was observed in response to BPP-5a not included in β-CD. Our findings suggest that the strategy of inclusion of BPP-5a in β-CD can be used to prepare BPP formulations with oral activity.

4. Conclusion

In this work, a new pharmaceutical formulation based on the brakykinin potentiating penta peptide (BPP-5a) complexated with β-CD has been described. Physical–chemical analyses allowed to characterize the BPP-5a/β-CD complex, where the β-CD cavity preferentially recognize the tryptophan hydrophobic residue of the peptide and the peptide exhibits a greater rigid structure upon complexation. Thermodynamic data showed weak interaction suggesting easy dissociation in biological systems. *In vivo* experiments in spontaneously hypertensive rats allowed to verify that the reduction in blood pressure, of the new formulation, could be attributed to the increase of the biodisponibility of the BPP-5a, caused by the peptide inclusion in β-CD.

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