Journal of Peptide Science, Vol. 1, 227–235 (1995)

Characterization of the Conformational Domains of Bradykinin by Computational Methods

JUAN J. PEREZ[1], YOLANDA M. SANCHEZ[2] and NURIA B. CENTENO[1]

[1]Department d'Enginyeria Quimica, Universitat Politecnica de Catalunya, Barcelona, Spain

[2]Department de Quimica Organica, Universitat de Barcelona, Barcelona, Spain

Received 15 October 1994
Accepted 23 December 1994

Abstract: The AMBER 4.0 force field was used to perform a characterization of the conformational profile of the nonapeptide bradykinin. A thorough conformational search was carried out using molecular dynamics as sampling technique, by computing cycles of high (900 K) and low (300 K) temperature trajectories. A total of 2400 minima were generated and subsequently clustered using the root-mean-square of the backbone dihedral angles as criterium. After the use of a tolerance value of 20°, the conformations were clustered in 233 unique conformations with energies up to 40 kcal/mol above the lowest minimum. The analysis of the low-energy conformations indicate that the peptide exhibits a high tendency to adopt a β-turn at the C-terminus and a propensity to adopt a bent structure at the N-terminus. These results are in agreement with the experimental evidence reported in the literature and provide detailed information necessary to understand the conformational preferences of the peptide.

Keywords: Bradykinin; conformational domains; conformational search; molecular mechanics

Abbreviatons: BK, bradykinin; MD, molecular dynamics; r.m.s., root-mean-square; CD, circular dichroism

INTRODUCTION

Bradykinin (BK) is an endogenous peptide of sequence (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) released from a protein precursor, kininigen, in response to inflammation, trauma, burns, shock, allergy and some cardiovascular diseases. The release of the peptide results in increasing vascular permeability, tissue swelling, redness and pain, and it has been associated with the symptoms of the common cold [1–4].

The characterization of the bioactive conformation of BK has been focus of attention by several groups in the past [5–20]. Although a linear nonapeptide is expected to be very flexible, owing to the number of proline residues in BK, it can be thought that some ordering might prevail in the molecule as a consequence of the restriction of the corresponding ϕ angles. Early conformational studies of BK by circular dichroism (CD) [5–7], NMR [6–11] and laser Raman spectroscopy [6] did not reveal a preferred conformation of the peptide in solution, although it was pointed out that in dimethylsulphoxide (DMSO), the peptide was likely to exhibit a β-turn motif involving either residues 6–9 or 5–8 [6]. More recently, in an attempt to carry out the conformational study of the peptide in an environment that mimicked physiological conditions, a 2D-NMR analysis of the peptide was performed in sodium dodecyl sulphate micelles [12]. This study reported evidence consistent with a β-turn involving residues 6–9. Similar conclusions were also drawn from an NMR

Address for correspondence: Juan J. Perez, Department d'Enginyeria Quimica, UPC, Av. Diagonal 647, E-08028 Barcelona, Spain.

CCC 1075-2617/95/040227-09

study of the peptide in a mixture of 9:1 dioxane-d6: water [13]. These results were further confirmed by a recent 2D-NMR study of the peptide in DMSO, 9:1 dioxane: water and in lyso phosphatidylcholine micelles [14].

The previous studies strongly support a preference of the peptide to adopt a β-turn involving residues 6–9 in various solvents. However, not all the results reported in the literature support this finding. Thus, results of a recent CD and NMR study of the peptide in trifluoroethanol/water solutions are consistent with a two conformation model: an all-*trans*, fully extended conformation and a bent conformation consisting of a type IV β-turn at the N-terminus [15]. Independently, other authors have suggested a conformational preference of BK for other secondary motifs, like a γ-turn between residues 6–8 [16], or a tight turn between residues 4–6 in the bioactive conformation [17].

In addition to the different spectroscopic techniques used in the structural characterization of a peptide, theoretical studies can be used to provide detailed structural information concerning the conformational preferences of the peptide. Very few theoretical studies have addressed this goal for BK. In a pioneering work, Galaktionov, *et al.* used the building up procedure to assess the accessible conformations of the peptide [18]. The authors concluded that the peptide exhibits preferably a pseudo-cyclic structure by interaction of the arginyl sidechain of [Arg^1] and the carbonyl oxygen of [Arg^9]. In a more recent effort to understand the structural implications of SAR studies on some BK analogues, molecular dynamics (MD) was used to suggest that the bioactive conformation of BK exhibits a tight turn between residues 4–6 [17]. In a recent study, using annealing molecular dynamics as the sampling technique, the conformational space of the peptide was explored [19]. In spite of the limited sampling, the results obtained agree well with the reported NMR studies by Lee, *et al.* [12], providing a computational support for a β-turn motif at the C-terminus. In a step further, in order to assess the conformational features of the bioactive conformation of BK, Kyle *et al.* [20] modelled the transmembrane region of its receptor and subsequently docked the peptide in it. The authors, using all the information available on receptor point mutations and assuming a β-turn at the C-terminus of the peptide, proposed a model consistent with most of the structural information reported in the literature on BK.

A thorough study of the conformational profile of a nine residue peptide, still represents a challenge for the computational methods. However, the need to understand the conformational features of BK prompted us to carry out a more complete conformational search than those presently available. We report in the present work the results of a thorough conformational search of BK using an iterative procedure that involves the use of molecular dynamics as the sampling technique. The results of this study will help to understand the conformational preferences of the peptide.

METHODS

All the calculations were carried out within the molecular mechanics framework using the all-atom AMBER 4.0 force field [21]. The peptide was studied in its zwitterionic form with the arginyl sidechains charged, so that the total charge of the peptide was +2. No explicit solvent was included in the calculations, although an effective dielectric constant of 80 was used to screen the electrostatic interactions and no cutoff was used.

The strategy used to search the conformational space consists of an iterative procedure that involves the use of MD as the sampling technique [22]. The procedure requires an initial library of conformations that are as much dissimilar as possible to each other. Each of the structures with energy lower than a prefixed threshold is considered as the starting conformation of a high-temperature MD calculation. During the computation of the trajectory, snapshots at regular time steps are taken and subsequently minimized. The new minima are added to the master list (library) of conformations. A subset of it, according to an energy criterion, is considered for a low-temperature MD run. The computation of high- and low-MD trajectories are alternatively continued until no new low-energy conformation is detected from an MD run.

In the present study, 200 structures were generated by random assignment of the backbone torsional angles. Each structure was then minimized using 100 cycles of steepest descent, followed by conjugate gradient minimization until a convergence of 0.001 on the root-mean-square (r.m.s.) was achieved. The total of 200 structures were ranked ordered and checked for similarities in backbone torsions. Two conformations were considered unique if the r.m.s. of the backbone dihedral angles were greater than 20°.

Of this initial set, only a subset of conformations is

considered for MD calculations, selected according to an energy criterion. Each of the conformations is the starting point for a MD trajectory at high temperature. In the present case, each of the unique conformations within a 5 kcal/mol threshold in respect to the lowest energy structure were selected as starting geometries for a 75 ps MD trajectory at 900 K. Coordinates were stored every 1 ps and subsequently minimized. The resulting conformations were added to the previous 200 structures generating a master list of peptide minima.

Once the unique conformations had been selected, those within an energy threshold of 5 kcal/mol were chosen for a 50 ps MD calculation at 300 K. In this case coordinates were stored every 2 ps and subsequently minimized. The minimized structures were added to the library of minima and once again the unique conformations were selected.

In this step those within a threshold of 5 kcal/mol and generated from the 300 K cycle were selected for a 900 K MD run. The procedure of high- and low-temperature MD was continued until no new structures were found in the working set of conformers within a 5 kcal/mol cutoff selected for each cycle.

RESULTS AND DISCUSSION

Following the sampling procedure described in the methods section, convergency was achieved after nine cycles of high- and low-temperatures MD runs. The procedure generated a library of 2400 minima, with energies ranging from −4.5 to 35.0 kcal/mol. Of them, only 233 were considered unique by systematic comparison of the r.m.s. of the backbone dihedral angles as explained in the methods section.

Although there are more efficient methods to obtain the global minimum of a peptide [23, 24], the procedure used in the present study has the advantage of providing a good sampling of the low-energy conformations of the molecule in addition to the global minimum. This is important since we may be interested in characterizing the structural features of the bioactive conformation, which is not necessarily the global minimum in solution.

Figure 1 shows the distribution of the unique conformations at the end of the search. The histogram exhibits the typical profile found in conformational searches of flexible molecules, which indicates an adequate sampling [25, 26]. The profile to the left of the maximum of the distribution looks smoother than to the right, since the searching

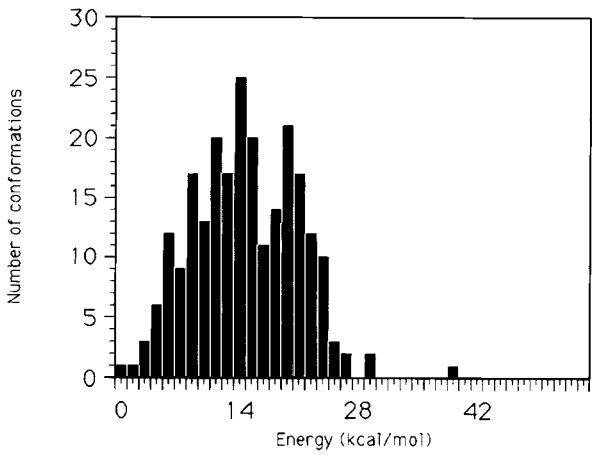

Fig. 1 Histogram of the energy distribution of 'unique' conformations of bradykinin of the present conformational search. Histogram bar width is 1.4 kcal/mol.

procedure is biased, enhancing the sampling of conformations of lower energy than the starting point. Since the highest probability for a starting conformation is to belong to the region of the maximum of the distribution, it will be expected that conformations to the left to this point (lower energy) are more adequately sampled than conformations with higher energy, and the distribution of minima in this region presents a better match to the standard density of states of a flexible molecule [25, 26].

All the conformations within a 5 kcal/mol threshold exhibit a globular structure and the backbone of most of them adopt a hairpin turn motif at the C-terminus. A few of them also exhibit a bend at the N-terminus region providing to the peptide either a global 'C' shape or a twisted 'S'. The arginyl side-chains of both termini also may play an important role on the global shape of the peptide by facing to the interior and forming diverse hydrogen bonds with carbonyl oxygens of the peptide backbone.

Backbone dihedrals of the 15 unique lowest energy conformations, as well as their energy relative to the lowest energy minimum, are shown in Table 1, whereas the hydrogen bonding scheme of each of these structures is listed in Table 2. For γ-turn only the middle residue involved is indicated, whereas for β-turns the two intermediate residues are listed. The rest of the hydrogen bonding interactions listed in Table 2 involve the moiety listed of the residue in the corresponding column, interacting with another moiety of a different residue explicitly indicated.

With the results of Tables 1 and 2 a simple analysis of the conformational motifs of the peptide can be done. Regarding the C-terminus, a type I

Table 1. Relative conformational energies, ΔE (in kcal/mol) and backbone dihedral angles (in degrees) of the 15 lowest energy conformations

Conf No.	1	2	3	4	5	6	7	8
ΔE	0.0	2.2	3.4	3.9	4.2	4.4	4.5	4.5
ψ_1	127	85	87	146	88	100	148	94
ω_1	−169	−176	177	179	173	167	171	175
ϕ_2	−76	−81	−76	−75	−58	−54	−74	−51
ψ_2	163	168	145	166	129	144	169	159
ω_2	175	171	−179	174	178	−179	174	−179
ϕ_3	−46	−54	−71	−51	−49	−45	−64	−71
ϕ_3	−35	159	−47	−49	−43	−52	−19	91
ω_3	171	−172	−174	177	180	180	171	−177
ϕ_4	−76	72	72	−91	−58	−66	−76	82
ψ_4	84	80	−88	64	−54	−47	−25	145
ω_4	−175	−164	177	176	180	−177	−177	175
ϕ_5	−81	−79	−150	−68	−66	−61	59	−72
ϕ_5	63	82	−49	−35	−61	142	65	−62
ω_5	174	180	−177	−178	−178	−178	180	161
ϕ_6	−81	−69	−67	67	−128	53	−76	−81
ψ_6	160	150	145	89	101	64	140	162
ω_6	176	−177	−177	5	178	−172	−169	177
ϕ_7	−51	−67	−71	−67	−45	−80	−70	−72
ψ_7	−48	−41	−43	146	113	70	−38	103
ω_7	179	−178	179	−170	−171	−179	−172	176
ϕ_8	−73	−68	−64	−50	−160	−169	−59	−150
ψ_8	−37	−40	−30	110	166	−40	−42	−41
ω_8	175	173	176	175	166	178	179	−171
ϕ_9	−90	−79	−77	55	179	−151	−104	−75

Conf No.	9	10	11	12	13	14	15
ΔE	4.9	5.3	5.5	5.6	6.0	6.1	6.3
ψ_1	144	84	99	135	79	90	104
ω_1	173	−178	−174	176	−171	175	173
ϕ_2	−75	−76	−77	−67	−79	−54	−57
ψ_2	148	164	139	166	152	111	157
ω_2	−176.	171	174	−174	−179	−169	180
ϕ_3	−71	−53	−46	−57	−74	−55	−66
ψ_3	153	−47	135.	−65	82	−71	−56
ω_3	−179	174	−176	−171	−171	−176	175.
ϕ_4	88	−74	75	−91	163	103	−74
ψ_4	−49	−163	68	−91	−36	47	157
ω_4	−178	−177	180	−177	179	−176	−177
ϕ_5	−84	−142	−157	−89	−134	−147	−76
ψ_5	54	103	−51	129	43	−50	85
ω_5	−178	173	175	167	172	177	175
ϕ_6	180	−71	−69	−114	−166	−144	−75
ψ_6	125	162	163	154	151	69	171
ω_6	−176	174	174	179	174	175	−176
ϕ_7	−82	−51	−51	−50	−61	−69	−52
ψ_7	61	−39	−50	−53	−23	109	129
ω_7	−176	−177	175	173	168	−178	−178
ϕ_8	−154	−96	−92	−68	−72	−64	48
ψ_8	−53	2	86	−38	89	−50	33
ω_8	−175	179	172	178	179	179	180
ψ_9	−163	−138	61	74	−150	−78	−144

Table 2 Hydrogen Bond Network of the 15 Lowest Energy Conformations[a]

	Arg^1	Pro^2	Pro^3	Gly^4	Phe^5	Ser^6	Pro^7	Phe^8	Arg^9
1	sc...CO[Ser^6] sc...COterm			γ-turn	γ-turn		β-turn	β-turn	
2	sc...CO[Ser^6] sc...CO[Pro^2]				γ-turn		β-turn	β-turn	
3	sc...CO[Pro^7] sc...CO[Phe^8]			γ-turn			β-turn	β-turn	
4			CO...NH[Arg^9]	γ-turn					
5		CO...NH[Ser^6]							
6	sc...CO[Ser^6] sc...COterm					CO...NH[Phe^5] sc...COterm	γ-turn		
7	sc...COterm		β-turn	β-turn		sc...sc[Arg^9]	β-turn	β-turn	
8			γ-turn						
9				NH...COterm	γ-turn				
10							β-turn	β-turn	
11					NH...COterm		β-turn	β-turn	
12							β-turn	β-turn	
13			γ-turn					γ-turn	
14		CO...NH[Ser^6]							
15	sc...CO[Gly^4]				γ-turn		β-turn	β-turn	

[a] γ and β turns are listed by the middle residues involved in the turn. In the other cases, the interaction should be understood between the group of the corresponding residue of the column and a moiety, which residue is explicitly stated in brackets. 'sc' refers to 'side chain'.

β-turn involving residues 6–9 is a relatively common motif, exhibited by conformations 1, 2, 3, 7, 10, 12 and 15. Regarding the N-terminus, only conformations 7 and 13 exhibit a type I β-turn between residues Pro^2 and Phe^5. There are also other motifs found. Thus, conformations 1, 2, 9 and 15 exhibit a γ-turn involving residue Phe^5, conformations 1, 3 and 4 involving residue Gly^4, conformations 6 and 9 involving Pro^7 and conformations 8 and 13 involving residue Pro^3. Finally, as can be seen from Table 2, arginine and serine sidechains play an important role in stabilizing the globular structure of the peptide. Figure 2(a) depicts pictorially the global minimum and Figures 2(b)–2(h) some low-energy conformations that exhibit different motifs, selected according to the results of Table 2.

Comparison of the present results with available experimental data or other theoretical investigations is very interesting. The simple Chou–Fasman [27] procedure yields high β-turn probabilities to sequences Pro-Pro-Gly-Phe and Ser-Pro-Phe-Arg. As was mentioned in the Introduction, the presence of the latter conformational feature has definitely been confirmed in solution by NMR techniques [6, 12–14] and assigned preferably as a type I β-turn [13]. A β-turn between residues 6–9 is observed in almost 50% of the low-energy conformations listed in Table 1.

In a previous theoretical study [19] using annealed molecular dynamics as the sampling technique, some accessible conformations of BK were reported and compared with the structures reported by NMR studies. The authors carried out the calculations on the zwitterionic form of the peptide and with the arginyl sidechains charged, using a dielectric constant of 1 ($\varepsilon = 1$). These structures were also reminimized with $\varepsilon = 4$ and $\varepsilon = 25$. Final low-energy structures (1, 21, 33, 36, 40 and 41) of [18] were kindly provided to us by the authors and analysed. These structures are dominated by the strong electrostatic interactions between point charges, and according to the results of Table 3 of [18] some of them are no longer minima when they are reminimized with higher dielectric constants. It is known that the use of a small dielectric constant can yield spurious minima, owing to the dominating electrostatic interactions mainly if the model of the peptide is charged.

This point reflects one of the main differences with present calculations. Because of the high dielectric constant used in the present work, the electrostatic

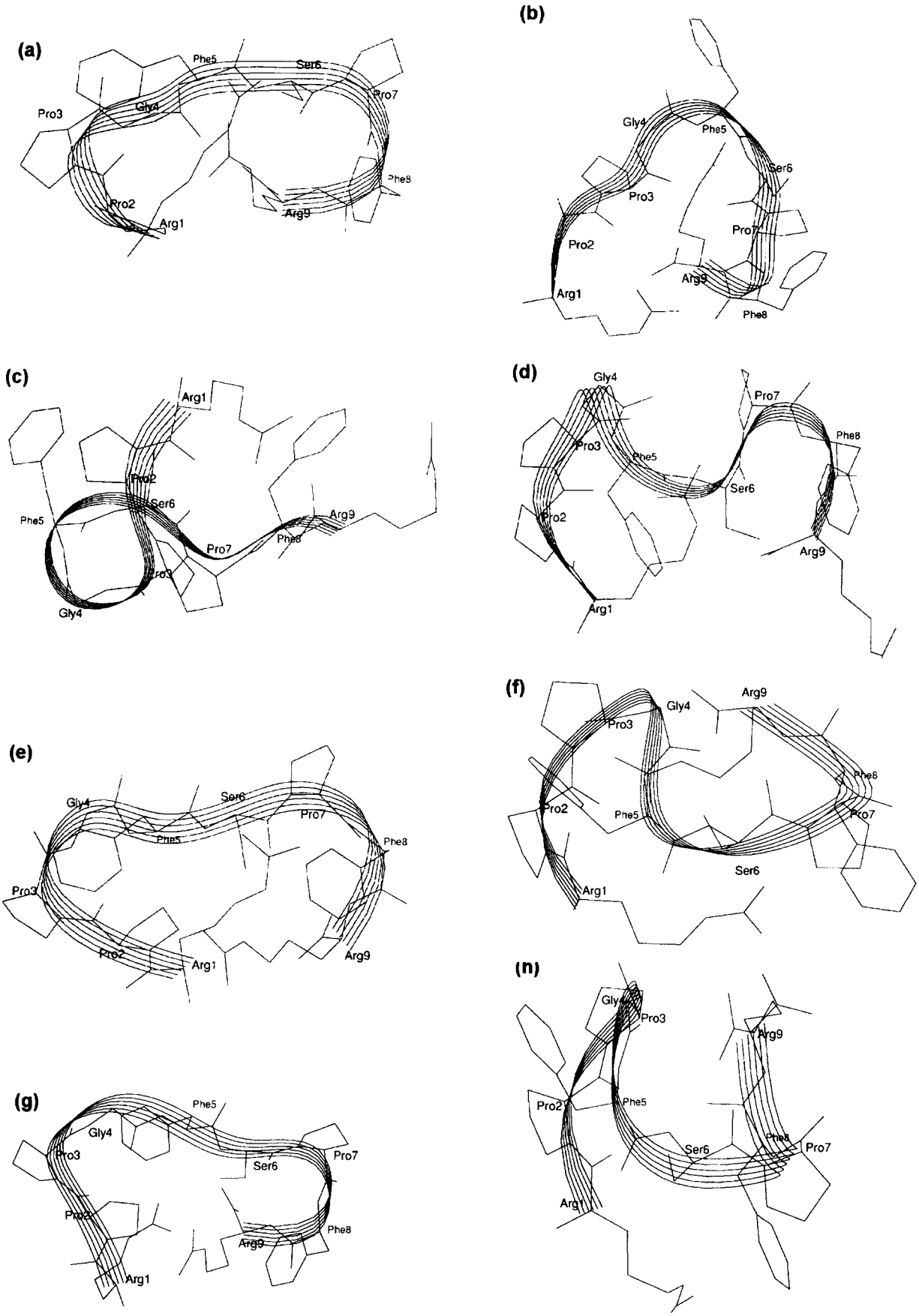

Fig. 2 Ribbon representation of selected low-energy conformations of bradykinin exhibiting different conformational motifs (see Table 2). Hydrogens have been omitted for clarity: (a) global minimum; (b) conformation no. 3; (c) conformation no. 5; (d) conformation no. 6; (e) conformation no. 7; (f) conformation no. 9; (g) conformation no. 10; (h) conformation no. 13.

interactions are screened to a level that their influence is balanced with the rest of the terms included in the force field. Previous studies demonstrate that the conformations found for the zwitterionic form with a high dielectric constant are equivalent to those found for the uncharged peptide with a low dielectric [22, 28]. Thus, we only analysed conformations 40 and 41 of [18] with present results first because they are the low-energy conformations that were retained after reminimization with a higher dielectric constant, and second because of their similarity with the conformations proposed by NMR studies [12]. Comparisons were carried out by calculating the r.m.s. of the α-carbon trace of residues 2–9, once the two molecules were optimally superimposed. With this criterion, it can be considered that both conformations are present in our library of low energy structures. Conformation no. 40 exhibits the highest similarity with our conformer no. 2, with an r.m.s. of 1.58 Å and conformation no. 41 exhibits a minimum r.m.s. value of 1.24 Å with our conformation no 10. Both structures have in common a β-turn motif between residues 6 and 9.

Regarding a β-turn involving residues 2–5, only a recent work [15] provides experimental evidence supporting it. In our calculations, of the low-energy structures, only conformations 7 and 13 exhibit clearly a β-turn in this region. However, most of the conformations are bent at this terminus owing to the interaction of the Arg^1 sidechain with diverse backbone carbonyl oxygens, including the C-terminus carboxyl group, or the Pro^2, Ser^6, Pro^7 and Phe^8 carbonyls (see Table 2). This result was also observed in the study reported in [19]. Furthermore, these results were successfully used to design a cyclic analogue of BK [31, 32].

Several of the low-energy conformations exhibit γ-turns. This is very interesting, since they have been implicated as recognition motifs by the receptor [16, 17]. Thus conformations 6 and 9 exhibit a γ-turn involving Pro^7, whereas conformations 1, 2 and 9 exhibit a γ-turn involving Phe^5. Interestingly, the latter motif is compatible with a β-turn at the C-terminus of the peptide. These results provide some clues in order to ascertain the characteristics of the bioactive conformation of BK. However, the characterization of the bioactive form requires a careful comparison of the conformational profile of several peptide analogues with different pharmacological profiles and unfortunately cannot be deduced from a single study.

Finally, let us comment on the *cis/trans* isomerism of the peptide bonds due to the prolines present in the peptide. Prolines enhance the propensity of the previous peptide bond to acquire a *cis* conformation. In the present case, the lowest energy conformation exhibiting a *cis* peptide bond is only 3.9 kcal/mol above the global minimum at the Ser^6-Pro^7 (ω_6) peptide bond. There are several other conformations with this characteristic in the library of conformations generated. The first conformation exhibiting a *cis* ω_1 lies 12.8 kcal/mol above the global minimum and at 18.6 kcal/mol for the ω_2 dihedral. These results indicate that the ω_6 dihedral might be expected to be populated in a *cis* conformation since the conformation is energetically accessible, whereas the possibilities for ω_1 and ω_2 are much smaller, in agreement with the results of NMR studies [6–14].

CONCLUSIONS

Molecular mechanics calculations have been carried out to characterize the conformational profile of BK. A thorough conformational analysis was carried out using MD as the sampling technique. The results show that the peptide exhibits a high tendency to form a type I β-turn at the C-terminus. These results are consistent with several experimental observations. The presence of a tight turn involving residue 4–6 suggested from MD calculations seems to be compatible with the existence of a β-turn at the C-terminus. Present results suggest that the peptide also exhibits a bent structure at the N-terminus. To make this hypothesis compatible with the previous ones, this should be via hydrogen bonding of the Arg^1 sidechain with carbonyl groups of the backbone. These results are consistent with a 'C' type conformation of the peptide. Further studies are, however, necessary to characterize the bioactive conformation of BK fully. Comparison of the conformational profiles of the different analogues will be very helpful in determining the characteristics and the spacial arrangement of the moieties involved in recognition and activation of BK. Work in this direction is currently being carried out in our laboratory [32].

Acknowledgements

Y.M.S. acknowledges financial support from the Generalitat de Catalunya through the CIRIT for financial support. N.B.C. acknowledges financial support from the Department d'Ensenyament de la Generalitat de Catalunya. The authors wish to

express their gratitude to the Centre de Càlcul de l'EUPVG and CESCA for the computer time.

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