Possible conformations involved in the binding of neurotensin, xenopsin and bradykinin molecules to mast cell receptors

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1. INTRODUCTION

The study of binding of neurotensin molecule (NT, tridecapeptide pGlu[1]–Leu[2]–Tyr[3]–Glu[4]–Asn[5]–Lys[6]–Pro[7]–Arg[8]–Arg[9]–Pro[10]–Tyr[11]–Ile[12]–Leu[13]), of its analogues and fragments, as well as of several other peptide bioregulators to rat mast cells [1–3] has shown that xenopsin (XE, octapeptide pGlu[1]–Gly[2]–Lys[3]–Arg[4]–Pro[5]–Trp[6]–Ile[7]–Leu[8]) and bradykinin (BK, nonapeptide Arg[1]–Pro[2]–Pro[3]–Gly[4]–Phe[5]–Ser[6]–Pro[7]–Phe[8]–Arg[9]) compete successfully with neurotensin for the binding, whereas 15 other peptides are devoid of this property [2]. A comparable and relatively high (K_d~100 nM) level of specific binding to the same type of receptors observed for different peptides is a rather unusual example of peptide ligand–receptor interaction; it is legitimate to assume that one of the reasons responsible for such an effect is the similarity shared by some primary sequence elements and especially 'binding conformations' of NT, XE and BK molecules. The data concerning the three-dimensional structure of NT, XE and BK molecules necessary for their comparison can be provided by means of energy calculations; it must be noted that no experimental evidence as to the conformations of NT and XE molecules is available so far.

2. METHODS AND RESULTS

The calculated sets of low-energy backbone structures for XE and BK have been described in [4] and [5], respectively. Given below are the calculation results obtained for the NT molecule; the calculation procedures and the potential functions used were as in [4–7]. The general calculation pattern was essentially as for α-MSH molecule in [6]. During the first two steps, the 'model decapeptide' Glu[4]–Ala[5]–Lys[6]–Pro[7]–Arg[8]–Arg[9]–Pro[10]–Ala[11]–Ala[12]–Ala[13] containing all ionogenic groups of the molecule and the N-terminal pentapeptide pGlu[1]–Leu[2]–Tyr[3]–Glu[4]–Asn[5] were considered. Finally energy calculations were performed for all possible combinations of low-energy backbone structures for the two fragments overlapping in positions 4 and 5 of the peptide sequence, conformations of the Glu[4] sidechain being the same as determined according to the 'model decapeptide' calculations. This final step (energy calculations for ~410 structures of the whole NT molecule) was accompanied by the refinement of side chain packing for Leu[2], Tyr[3], Asn[5], Tyr[11], Ile[12] and Leu[13] residues by means of a specially designed algorithm [8].

The calculation resulted in 5 types of low-energy backbone structures found for the 4–13 fragment, which with optimal space orientation of the 1–3 fragment meet the requirement $\Delta U = U - U_{min} \lesssim$ 10 kcal/mol. These structures are listed in table 1. Keeping in mind that the 9–13 fragment possesses the same level of affinity to rat mast cell receptors as NT molecule itself [3], one can note that only 4 backbone structure types of this fragment are presented in table 1, namely BRBLL, BRRBL, BBLLB and BRBLB.

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Table 1

Low-energy structures of neurotensin

Residue	Angle	Molecular backbone conformation[a]				
		1	2	3	4	5
pGlu[1]	ψ	−32	151	151	−36	146
Leu[2]	ϕ	−112	−123	−119	−138	−133
	ψ	141	133	132	129	131
	χ_1	−77	−161	−161	−160	−160
	χ_2	98	80	81	100	100
Tyr[3]	ϕ	53	−132	−134	−137	−136
	ψ	110	139	142	137	133
	χ_1	165	−58	−61	−60	−60
	χ_2	−101	−80	−81	−80	99
	χ_3	100	−80	−80	80	80
Glu[4]	ϕ	44	46	51	56	56
	ψ	34	32	29	51	53
	χ_1	−75	−77	−77	−159	−159
	χ_2	−174	179	−177	−145	−144
	χ_3	−96	−99	−97	120	138
Asn[5]	ϕ	42	37	40	43	43
	ψ	62	71	62	37	38
	χ_1	−164	−60	−60	60	59
	χ_2	81	−80	−80	80	81
Lys[6]	ϕ	−129	−136	−135	−148	−147
	ψ	132	130	131	140	133
	χ_1	−78	−79	−75	−77	−75
	χ_2	154	161	163	161	162
	χ_3	−178	−166	−164	−159	−158
	χ_4	171	176	175	79	77
Pro[7]	ψ	−35	−46	−37	−102	104
Arg[8]	ϕ	−114	−133	−128	−140	−138
	ψ	129	127	129	153	141
	χ_1	−141	−139	−148	−77	−76
	χ_2	−91	−94	−107	160	154
	χ_3	112	104	109	−164	−168
	χ_4	139	88	92	−161	179
Arg[9]	ϕ	−123	−128	−122	−137	−177
	ψ	151	149	137	138	127
	χ_1	−57	−53	−57	57	43
	χ_2	173	172	152	−166	−152
	χ_3	−65	−61	−66	−179	−179
	χ_4	−86	−94	−98	116	140
Pro[10]	ψ	−28	−24	−15	127	−31
Tyr[11]	ϕ	−130	−127	−99	60	−115
	ψ	146	137	−44	134	145
	χ_1	43	61	−4	−60	40
	χ_2	85	97	101	100	82
	χ_3	99	100	80	80	80
Ile[12]	ϕ	39	37	−135	45	33
	ψ	95	98	98	96	90
	χ_1	−49	−58	56	−59	−42
	χ_2	122	100	120	100	120
Leu[13]	ϕ	48	50	26	−96	−104
	ψ	91	96	93	140	145
	χ_1	−78	−161	−79	−80	−178
	χ_2	99	100	100	100	81
ΔU(kcal/mol)		0.0	0.41	10.12	10.21	10.28

[a] Symbols B, R and L are used to denote local energy minima of the peptide backbone in B ($\phi \sim -140°$, $\psi \sim 140°$), R ($\phi \sim -60°$, $\psi \sim -60°$), L ($\phi \sim 60°$, $\psi \sim 60°$) and H ($\phi \sim 80°$, $\psi \sim -80°$) regions of potential maps: (1) RBLLLBRBBRBLL; (2) BBBLLBRBBRBLL; (3) BBBLLBRBBRRBL; (4) RBBLLBBBBBLLB; (5) BBBLLBBBBRBLB

3. DISCUSSION

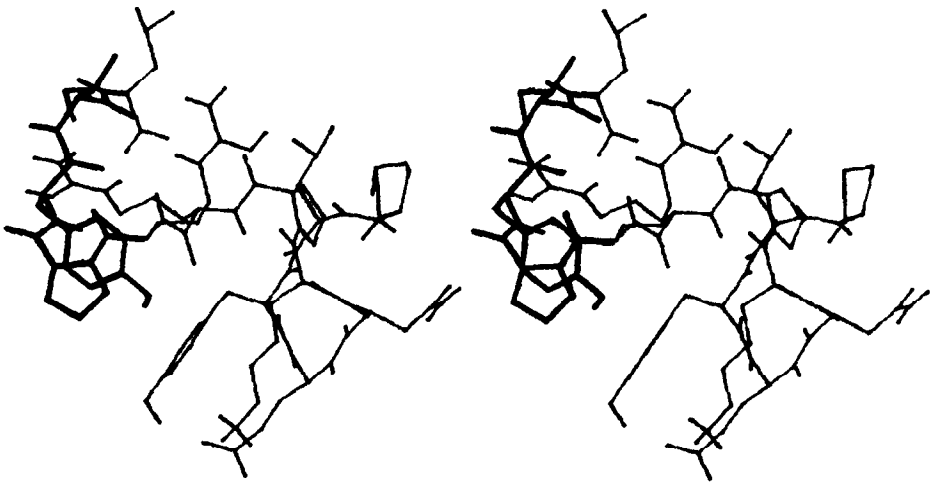
The 9–13 fragment responsible for the binding of NT molecule to mast cell receptors contains a ...Pro–X... sequence (X is an aromatic amino acid residue) which is common for NT, XE and BK molecules (see table 2). The data shown in table 2 allow us to assume that the possible 'binding site' for NT molecule can be restricted to the 9–12 fragment only, since the shortening of the peptide chain due to omission of one C-terminal residue (BK) affects the affinity only insignificantly whereas that involving two residues (AT) leads to a complete lack of affinity. On the other hand, our previous energy calculations for XE [4] and BK [5] indicate that the 'binding site' conformations of

Table 2

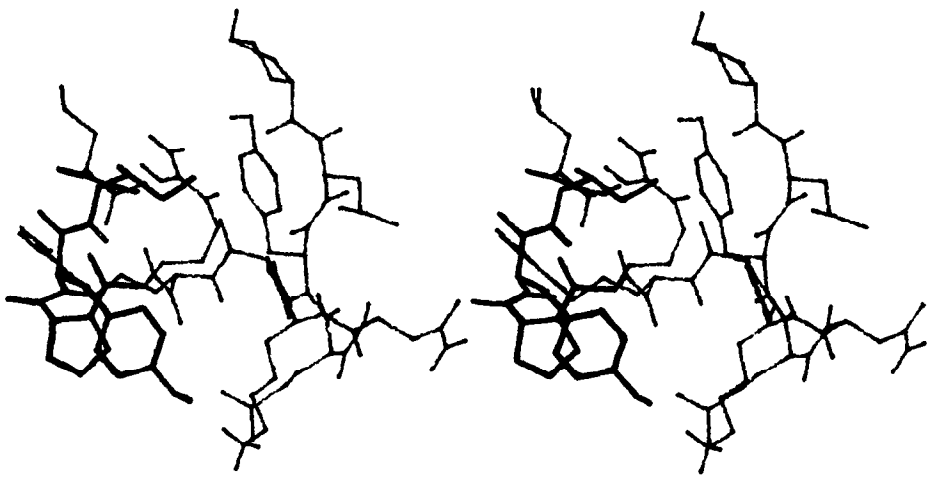
Comparison of amino acid sequences of NT, XE, BK and AT molecules

Neurotensin	(NT)	pGlu–Leu–Tyr–Glu–Asn–Lys–Pro–Arg–Arg–Pro–Tyr –Ile–Leu
Xenopsin	(XE)	pGlu–Gly–Lys–Arg–Pro–Trp –Ile–Leu
Bradykinin	(BK)	Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe –Arg
Angiotensin[a]	(AT)	Asp–Arg–Val–Tyr–Ile–His–Pro–Phe

[a] The only one among 15 peptides discussed in [2] (NT, XE and BK are not included) having a Pro–X sequence in its molecule



a



b

Fig.1. A stereo-view of neurotensin structures 1 and 3 (table 1): (▬), site in the molecule directly involved in the binding to rat mast cells; (a) conformation of RBL type; (b) conformation of RRB type.

Table 3

Comparison of the complete sets of low-energy backbone conformations for NT, BK [5] and XE [4] molecules

NT:	RBLLLBRB BRBL L	*
	BBBLLBRB BRBL L	*
	BBBLLBRB BRRB L	**
	RBBLLBBB BBLL B	
	BBBLLBBB BRBL B	*
BK:	BBBHB BRBB	
	BBRRR BRBL	*
	BBBHB BRRB	**
	BBBHR BRBB	
	BBRRR BBBB	
	BBBHR BRRB	**
	BBBHB BRRL	
	BBBHR BRBL	*
	BBRRB LBBB	
	BBBHR BBLB	
	BBRRB LRBB	
	BBRLR BBBB	
XE:	BRB BRBL L	*
	BHR BRBL B	*
	BRB BBLL B	
	BRB BRRB L	**
	BBL LRRB B	
	RRB LRRB B	
	BRB BRBL B	*

By one and two asterisks are marked conformations with the three-dimensional structure of the 'binding site' in all of three sets of low-energy backbone conformations

BRBL and BRRB types appear to be common for all three peptides in question (table 3).

However, the biological data obtained for various NT analogues with single replacements for amino acid enantiomers demonstrate that only analogues with replacements in positions 10–12 exhibit a decreased level of affinity to receptor [3]. Therefore, the backbone conformation of B type in position 9 (which is sterically hindered for D-Arg residue) cannot represent the 'conformational' factor affecting the tightness of ligand–receptor binding. Thus, it may be concluded that tripeptides Pro^{10}–Tyr^{11}–Ile^{12} (NT), Pro^{5}–Trp^{6}–Ile^{7} (XE) and Pro^{7}–Phe^{8}–Arg^{9} (BK) can possibly be the fragments providing a certain complementarity of NT, XE and BK molecules to the 'common' binding site of rat mast cell receptors, their common 'binding conformation' being that of RBL or RRB type (fig.1a,b). It can be added finally, that the general shape of the two proposed 'binding conformation' types are essentially the same (fig.1): they differ only in the twist by ~180° of the peptide bond plane connecting the second and third residues of the binding site of the molecule.

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