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THREE-DIMENSIONAL RECOGNITION REQUIREMENTS FOR ANGIOTENSIN AGONISTS: A NOVEL SOLUTION FOR AN OLD PROBLEM

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Summary: A novel approach involving the search for a common spatial arrangement of functionally important (pharmacophoric) groups in low-energy conformers of AT and its active analogs has been employed to determine the receptor-bound ("biologically active") conformation(s) for angiotensin II (Asp^1-Arg^2-Val^3-Tyr^4-Val/Ile^5-His^6-Pro^7-Phe^8, AT). The four pharmacophoric groups for AT are the aromatic moieties of Tyr^4, His^6 and Phe^8 residues, as well as the C-terminal carboxyl. Geometrical comparison of the sets of low-energy backbone conformers for AT itself, and two analogs, [(αMe)Phe^4]-AT and [Pro^5]-AT, yielded the model for the receptor-bound conformation(s), which is compatible with cyclic AT analogs possessing substantial binding to specific AT receptors. A new analog, [D-Tyr^4, Pro^5]-AT, was designed based on the proposed receptor-bound conformation. The analog showed a good affinity (IC_{50} = 42.8 nM) towards specific AT receptors.

The efforts to determine a receptor-bound ("biologically active") conformation(s) for angiotensin II (Asp^1-Arg^2-Val^3-Tyr^4-Val/Ile^5-His^6-Pro^7-Phe^8, AT) were initiated more than twenty years ago. Both molecular modeling and physico-chemical measurements were involved (for exhaustive list of references up to 1988 see [1]), and different models of the AT receptor-bound conformation were proposed. Among them were, for instance, those with an inverted γ-turn at the Tyr^4 residue suggested by Printz *et al.* [2], and with a β-III-like turn at the Tyr^4-Val^5 residues (i.e., with a right-handed α-helical type structure for Tyr^4). The latter model was proposed by Marshall *et al.* (see [3] for final model) based on biological activity of [(αMe)Phe^4]-, [Pro^5]- and [Pro^3]-AT analogs, and, independently, by Nikiforovich *et al.*, [4,5] based on biological activity data for series of aza-α'-containing analogs of AT. Recently, several types of cyclic AT analogs with conformational restrictions have been found to possess high affinity towards AT specific receptors [6,7]. However, the conformations available to the set of active analogs are not consistent with a unique model for the backbone of the AT receptor-bound conformation(s). Whereas backbone conformations of *cyclo*[Sar^1, HCys^{3,5}]-AT seem to be compatible with a γ-turn or right-handed α-helical structures for Tyr^4, both these conformers are sterically hindered for *cyclo*[Sar^1, Cys^3, Mpt^5]-AT, while Tyr^4 backbone conformations for this

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analog are, in turn, incompatible with those of *bis-cyclo*[Sar^1, Mpc^3, Cys^5] parallel and antiparallel dimers (see [7] for detailed discussion).

To resolve this apparent contradiction, an alternate approach involving the search for a common spatial arrangement of functionally important (pharmacophoric) groups in low-energy conformers of AT and its active analogs, rather than comparison of dihedral angle values for the peptide backbone (comparison "at the level of the Ramachandran map" [8,9]), has been used. The approach has previously yielded a model of the receptor-bound conformation for δ-opioid peptides [10], which was then employed for designing new cyclic δ-selective analogs [11, 12]. One of the prerequisites for the successful use of this approach is *a prior* knowledge of which molecular groups are functionally important for recognition and activation. Fortunately, in the case of AT agonists, it is commonly accepted that four groups are indispensable for optimal triggering of the biological response, the aromatic moieties of Tyr^4, His^6 and Phe^8 residues, as well as the C-terminal carboxyl (e.g. [13]).

METHODS

The methods for conformational search and energy calculations were essentially the same as described previously for other peptides (e.g. [10]). The ECEPP potential field [14] was used for conformational energy calculations assuming rigid valence geometry with planar *trans*-peptide bonds (both *trans* and *cis* peptide bonds were examined for Pro residues; in these cases the ω angle also was allowed to rotate). The valence geometry of the (αMe)Phe residue was derived from crystal data on α-methylalanine [15]; partial atomic charges were calculated by the use of the SYBYL program (the Del Re method). Aliphatic and aromatic hydrogens were generally included in united atomic centers of CH_n type; H^α-atoms and amide hydrogens were described explicitly. The build-up calculation schemes for all compounds in question are presented in Table 1. At the first step of the calculations, all possible combinations of local minima of *E, F, C, A* and *A** types (according to the notation in [16]) for the peptide backbone for each amino acid residue were considered. For Pro residues, minima of *F, C,* and *A* types were considered. The same minima were considered for N-terminal residues, and the minima of *E, C* and A^* types were considered for C-terminal residues. Two filters were used to eliminate conformers from further consideration. First, only the backbone structures selected at the previous step by $E - E_{min} < \Delta E = 10$ kcal/mol were considered at subsequent steps. Second, from the set of low-energy structures obtained at the previous step only those differing by more than 60° in at least one value of any backbone dihedral angle were selected for the next step. The dihedral angle values of side chain groups and of the terminal groups of the backbone were optimized at every step before energy minimization to achieve their most favorable spatial arrangements employing the algorithm described earlier [10].

Geometric comparison for a pair of conformers belonging to different analogs included an assessment of the best fit of the spatial arrangement for the atomic centers chosen to represent a fragment bearing the pharmacophoric groups of AT molecule, namely, the C^β-atom for Tyr^4 residue, the C^α- and C^β- atoms for Val^5 - Phe^8 residues and the carbon atom of C-terminal carboxyl. Two conformers were regarded as geometrically similar when the corresponding **rms** value was less than 1.0 Å.

RESULTS

The build-up procedure found 56 different low-energy backbone conformers for AT, 14 for [(αMe)Phe^4]-AT and 46 for [Pro^5]-AT (see Table 1). None of them contain a *cis*-peptide group preceding the Pro^7 residue. It is interesting to note that all low-energy conformers of

Table 1. Build-up procedure to determine low-energy backbone conformers for AT and its analogs

Compound	Sequence	Structures considered	Structures selected
AT	Ac-Val-Tyr-Val-His-Pro-NMe	1,500	125
	Ac-Arg-Val-Tyr-Val-His-Pro-Phe	1,875	28
	Asp-Arg-Val-Tyr-Val-His-Pro-Phe	84	56
[(αMe)Phe^4]-AT	Ac-Val-(αMe)Phe-Val-NMe	125	60
	Ac-Val-(αMe)Phe-Val-His-Pro-NMe	900	67
	Ac-Arg-Val-(αMe)Phe-Val-His-Pro-Phe	1,005	32
	Asp-Arg-Val-(αMe)Phe-Val-His-Pro-Phe	96	14
[Pro^5]-AT	Ac-Val-Tyr-Pro-His-NMe	750	367
	Ac-Val-Tyr-Pro-His-Pro-Phe	3,303	137
	Asn-Arg-Val-Tyr-Pro-His-Pro-Phe	2,055	46
clo[HCys^{3,5}]-AT	Ac-HCys-Tyr-HCys-NMe	125	44
	Ac-Arg-HCys-Tyr-HCys-His-Pro-NMe	3,300	145
	Asp-Arg-HCys-Tyr-HCys-His-Pro-Phe	1,305	40
[D-Tyr^4, Pro^5]-AT	Ac-Val-D-Tyr-Pro-His-NMe	750	244
	Ac-Val-D-Tyr-Pro-His-Pro-Phe	2,196	79
	Asp-Arg-Val-D-Tyr-Pro-His-Pro-Phe	1,185	156

[(αMe)Phe^4]-AT contain a β-III-like turn centered at the Val^3-(αMe)Phe^4 fragment, with corresponding dihedral angle values of $\phi 3 \approx -80°$, $\psi 3 \approx -40°$ and $\phi 4 \approx -60°$, $\psi 4 \approx -30°$. At the same time, none of the [Pro^5]-AT low-energy conformers contain negative values for the $\psi 4$ dihedral angle.

[(αMe)Phe^4]-AT and [Pro^5]-AT were shown earlier to possess 19% and 10%, respectively, of biological activity of AT *in vivo* [17,18]. ([(αMe)Tyr^4]-AT has 93% of AT biological activity [19].) This implies that among their low-energy conformers there are those similar to AT conformers and to each other. Geometrical comparison between each AT low-energy conformer *vs.* every low-energy conformer of [(αMe)Phe^4]-AT and [Pro^5]-AT performed as described in the *Methods* section revealed two types of possible backbone structures for AT 4-8 fragment, each of them meeting the requirement of **rms** ≤ 1.0 Å when compared to both analogs. These backbone conformers are presented in Table 2 together with corresponding

Table 2. Possible models for receptor-bound conformers of AT and its analogs

Compound	Structure type	Tyr/D-Tyr (αMe)Phe		Val/Pro/ HCys		His		Pro		Phe
		φ	ψ	φ	ψ	φ	ψ	φ	ψ	φ
AT	I	-81	-32	-86	-35	-138	83	-60	64	-138
	II	-127	160	-82	-31	-140	77	-60	73	-137
[(αMe)Phe^4]-AT		-58	-31	-87	-40	-132	85	-60	73	-138
[Pro^5]-AT		-107	166	-60	-27	-143	77	-60	73	-138
cyclo[HCys^{3,5}]-AT		-72	-37	-76	-27	-147	101	-60	61	-142
[D-Tyr^4, Pro^5]-AT		-123	163	-60	-31	-142	77	-60	73	-138

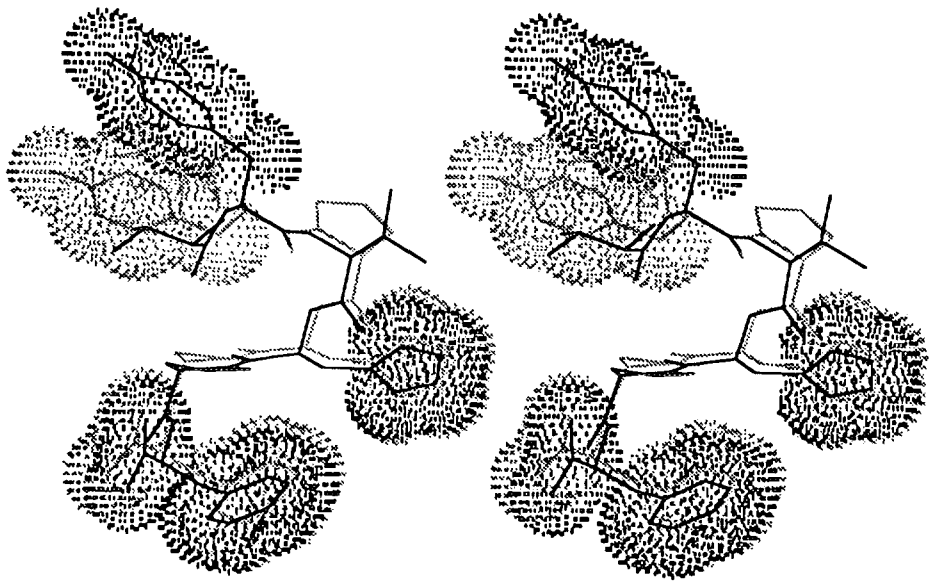

Fig. 1. Stereoview of AT structure of type I (in bold) overlapped on compatible [Pro^5]-AT structures. Only 3-8 fragments are shown. All hydrogen atoms are omitted. Van-der-Waals surfaces of functionally important groups are dotted.

conformers of [(αMe)Phe^4]- and [Pro^5]-AT. Both types of conformations represent the same three-dimensional structure of AT 5-8 fragment, differing in dihedral angle values for the residue in position 4. Obviously, the spatial structure of type **I** of AT 4-8 is very close to the structure of the [(αMe)Phe^4]-AT in $\psi 4$ value, whereas the spatial structure of type **II** is very close to the structure of [Pro^5]-AT. Nevertheless, the difference between the two in $\psi 4$ values does not affect significantly the spatial location of side chain aromatic moieties in position 4. Fig. 1 depicts an alignment between the spatial structures of type **I** for AT and the conformer of [Pro^5]-AT from Table 2. It is evident that the two Tyr^4 rings can occupy nearly the same spatial location for both molecules, with very similar spatial positions of the His^6 and Phe^8 side chains, as well as the C-terminal carboxyls. At the same time, the N-terminal parts of both molecules (exemplified in Fig. 1 by the Val^3 residues) are directed quite differently.

Since **the problem of the biologically active conformation(s) of AT and its analogs could not be resolved in terms of common backbone structures**, i.e., different kinds of turns, peptide chain reversals, *etc.*, it must be viewed in another way. We propose that any backbone three-dimensional structure that ensures a common spatial arrangement for the four important functional groups, such as that shown in Fig. 1, is a suitable candidate for the receptor-bound conformation of AT agonists. In this sense, the original model predicting the β-III-like turn at the Tyr^4-Val^5 region of AT [3-5] is quite appropriate (the AT conformation of type **I** in Table 2). At the same time, other spatial structures, like the AT conformation of type **II** in Table 2, are appropriate as well, as different backbone conformers can be accomodated at the receptor as long as the crucial residues are correctly positioned.

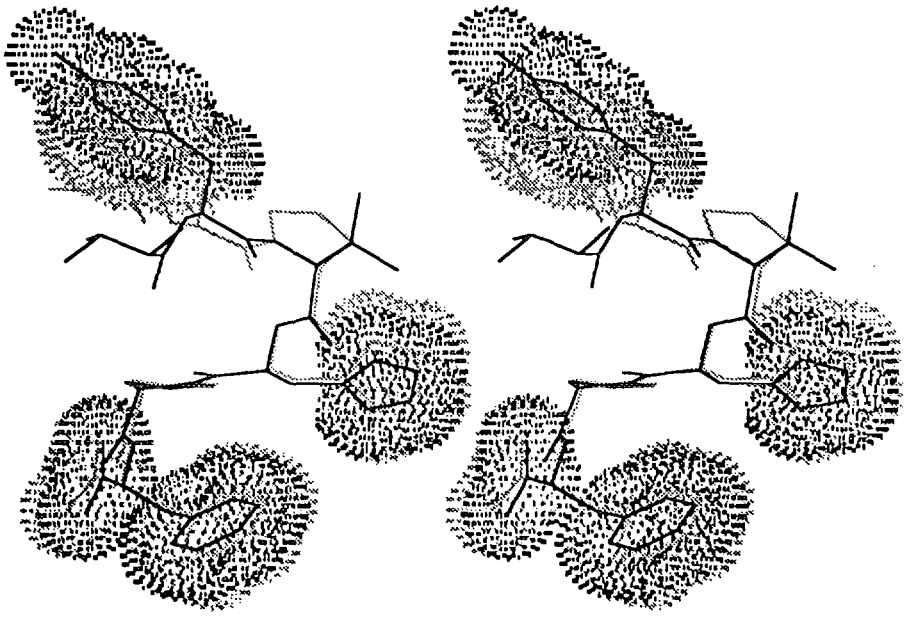

Fig. 2. Stereoview of AT structure of type I (in bold) overlapped on one of compatible [D-Tyr^4, Pro^5]-AT structures. Only 3-8 fragments are shown. All hydrogen atoms are omitted. Van-der-Waals surfaces of functionally important groups are dotted.

DISCUSSION

Energy calculations for cyclic analogs *cyclo*[HCys^{3,5}]-AT (Table 1) and *cyclo*[Sar^1, Cys^3, Mpt^5]-AT (to be published in detail later) found several low-energy backbone structures for both analogs, consistent with the spatial arrangement of the aromatic moieties for Tyr, His and Phe residues, and the carboxyl group, depicted in Fig. 1. Interestingly, for *cyclo*[HCys^{3,5}]-AT one can find low-energy conformers similar to the AT spatial structures of type **I** only (see Table 2); whereas for *cyclo*[Sar^1, Cys^3, Mpt^5]-AT, there are low-energy conformers compatible to both types of AT structures in Table 2. The reason is that the requirements of cycle closure force the N-terminal fragments of cyclic analogs to be oriented differently with regard to each other, whereas the C-terminal parts, including the Tyr^4 side chains, are similar. Thus, the novel approach applied in the present study can resolve the controversy concerning a unique receptor-bound conformation for cyclic analogs described in terms of dihedral angle values for peptide backbones [7].

The proposed spatial arrangement of important functional groups in AT analogs is similar to a model for AT conformation used by Samanen *et al.* to develop potent nonpeptidic antagonists [20]. However, in that model, the hydroxyl group of Tyr^4 side chain is close to the C-terminal carboxyl, which is not the case in our model. An antibody-bound conformation of AT revealed recently by X-ray techniques [21] also shows some resemblance to our model. At the same time, the side chains of Tyr^4 and His^6 in our model are too far apart to be consistent with a model by Moore *et al.* [22, 23], which suggests an intramolecular interaction between them. The

problem of proper orientation for AT side chains (i.e., possible values of χ_1 dihedral angles for Tyr, His and Phe) in receptor-bound conformation is not discussed here due to the lack of data on appropriately constrained analogs. Nonetheless, it should be mentioned that the backbone conformations presented in Fig. 1 are compatible with **g**$^-$ rotamers of Tyr^4 and Phe^8 side chains, and with **g**$^+$ or **t** rotamers of His^6 side chain for all AT agonists studied(to be published in detail later).

Comparison of proposed receptor-bound conformation(s) with those observed experimentally in solution is not straightforward and should be performed rather cautiously. However, it is noteworthy that our model is in general agreement with NMR data on spin-labeled angiotensin in water [24] that were reconfirmed recently [25]. On the other hand, clustering of the all three aromatic rings, deduced using the NMR data for [Sar^1]-AT in DMSO [26] seems unlikely from our model.

Finally, it was suggested based on Fig. 1, that the AT conformer of type **I** could be matched even more closely by an analog, which still contains the Pro^5 residue, but with D-Tyr^4 instead of Tyr^4. Energy calculations for [D-Tyr^4,Pro^5]-AT found 156 low-energy backbone structures (Table 1), many of them being compatible with the proposed receptor-bound conformation of AT (see Table 2 and Fig. 2). The analog was synthesized and tested for binding to rabbit aorta preparations. The IC_{50} for it was 42.8 nM, which could be equivalent to *ca.* 6% of the AT binding level (IC_{50} = 2.5 nM for AT [7]). This preliminary estimate is in the same range as the activity of [Pro5]-AT, which allows us to regard [D-Tyr^4,Pro^5]-AT as active analog, despite the absence of biological assay data. It is noteworthy that [D-Tyr^4]-AT had only 0.06 % of AT binding to tissue from rat ascending colon [27]. Thus, we were able to design a new and rather unusual AT analog on the basis of the three-dimensional model for AT recognition proposed in this study.

CONCLUSIONS

1. The three-dimensional recognition requirements for AT and its agonistic analogs were deduced employing the comparison of a common spatial arrangements for aromatic groups of Tyr^4, His^6 and Phe^8, as well as the C-terminal carboxyl, but not by comparison of dihedral angle values for the peptide backbones.

2. Correspondingly, the most important structural feature for binding and triggering the biological response is the conformation of the AT fragment 4-8 only, but not that of the entire AT molecule.

3. The models of the receptor-bound conformation for AT proposed in this study are compatible with cyclic AT analogs possessing substantial binding to specific AT receptors. A new analog with good affinity for the AT_2 receptor was designed based on the receptor-bound conformation.

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