Journal of Computer-Aided Molecular Design, 7 (1993) 173–182
ESCOM

J-CAMD 193

Stereochemistry of charged nitrogen–aromatic interactions and its involvement in ligand–receptor binding

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Received 2 October 1992
Accepted 16 November 1992

Key words: Crystal-structure statistics; Ligand–receptor complexes; Geometrical analysis

SUMMARY

Recently, new evidence was found for the involvement of charged nitrogen–aromatic interactions in ligand–receptor binding. In this study we report two favourable orientations of a phenyl ring with respect to a $R–N^+(CH_3)_3$ group, based on crystal structure statistics from the Cambridge Structural Database. In the first orientation, the phenyl ring is situated in between the substituents at about 4.5 Å from the nitrogen atom, and the ring is approximately oriented on the sphere around the nitrogen atom. In the second orientation, the phenyl ring is situated in the same direction as one of the N–C bonds at about 6.0 Å from the nitrogen atom, and the ring is tilted with respect to the sphere around the nitrogen atom. The same two orientations were also found in the crystal structures of three ligand–receptor complexes, which implies that these orientations probably play a major role in molecular recognition mechanisms.

INTRODUCTION

Many biologically active compounds, and in particular neuroactive compounds, carry an onium group which is thought to bind to one or more negatively charged amino acid residues at the active site of the specific receptor or enzyme. In the seventies Kier and Aldrich [1] and Höltje and Kier [2] indicated that aromatic residues at the active site might play a role in the binding of the onium group of a substrate as well, but little attention was paid to this view at the time. Recently, however, data from various disciplines indicate that these interactions are involved in ligand–receptor binding. In 1990 Dougherty and Stauffer [3] reviewed their own findings and those of other researchers on this subject. They put forward the hypothesis that the binding of the onium group of acetylcholine and related quaternary ammonium compounds to aromatic amino

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acid residues at a particular binding site might be at least as important as binding to a negatively charged amino acid. They based this hypothesis not only on theoretical and biochemical grounds but also on the results of binding experiments with synthetic receptors, mainly comprising aromatic rings [3,4]. These receptors showed a strong affinity for quaternary ammonium compounds.

Evidence to support the hypothesis of Dougherty and Stauffer was obtained from other sources as well. Alignment studies [5,6] on G-protein-coupled receptors, which have as their natural ligand a compound with a monoamine or quaternary ammonium group, showed that a number of aromatic amino acid residues are highly conserved. The importance of some of these residues in ligand–receptor binding has been demonstrated by site-directed mutagenesis [7,8] and photoaffinity labelling [9]. In the three-dimensional models of G-protein-coupled receptors proposed by Hibert et al. [10] in 1991, based on the electron cryomicroscopy structure of bacteriorhodopsin [11], three of these conserved aromatic residues are present at the proposed agonist binding site of the receptor. After the ligand molecule was fitted into the binding site, the onium group of the ligand appears to be surrounded by these three aromatic residues.

More support for the hypothesis has emerged from X-ray crystallography. As a first example we mention the crystal structure of immunoglobulin Fab McPC603 complexed with a phosphocholine molecule [12,13]. The striking feature of this complex is that the quaternary ammonium group of the ligand molecule makes shorter contacts with three aromatic amino acid residues than with two negatively charged amino acids. Other evidence is provided by the crystal structure of acetylcholinesterase (AChE), which was recently determined by Sussman et al. [14]. In this enzyme, the active site gorge leading to the catalytic triad is very rich in aromatic amino acid residues which – according to the authors – might channel the substrate molecule (acetylcholine) to its exact binding site by an 'aromatic guidance mechanism'.

Stereochemical information on charged nitrogen–aromatic interactions is of great interest for our understanding of molecular recognition mechanisms. In this paper we present the stereochemistry of the interaction between a phenyl ring and a $R–N^+(CH_3)_3$ group, based on crystal structure statistics from the Cambridge Structural Database (CSD) [15]. Burley and Petsko [16] have carried out similar studies on geometrical aspects of the interactions between side chains of aromatic amino acid residues and amino groups of other amino acids in protein structures. They used the Brookhaven Protein Data Bank [17] (PDB). Apart from the fact that the geometrical information we present here is more detailed, it is also more specific for one type of interaction. We used the crystal structures of three ligand–receptor complexes to check whether the stereochemistry we found in our CSD search is also present in ligand–receptor binding domains.

METHODS

The QUEST program of the CSD was used to obtain all crystal structures (R value less than 0.12; disordered and 'error-flagged' structures were omitted) containing both a $R–N^+(CH_3)_3$ fragment and one or more phenyl rings. These fragments were accepted if the N–C bond lengths for the onium group were between 1.3 and 1.7 Å and the C–N–C bond angles were between 95 and 125°, and if the C–C bond lengths for the phenyl rings were between 1.3 and 1.45 Å and the C–C–C–C torsion angles were between −10° and +10°. Only those crystal structures of which all phenyl rings and at least one onium group satisfied these criteria were used in our study. Phenyl

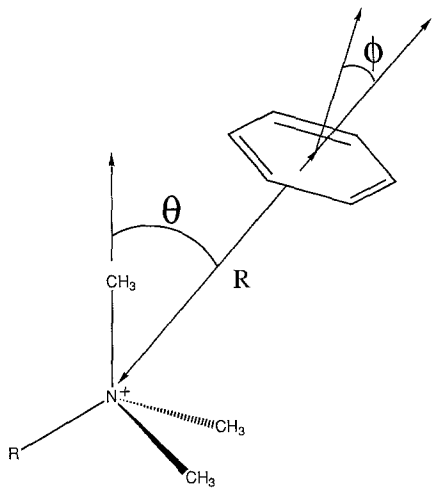

Fig. 1. Representation of the parameters, R, θ and ϕ, used to describe the position and orientation of a phenyl ring with respect to an onium group.

rings which were part of a larger aromatic system were also excluded. Most of the phenyl rings that were observed interacted intermolecularly with the onium group. Only those intramolecular interactions were considered where the nitrogen atom and the phenyl ring were separated by at least three bonds. The EUCLID package [18] was used to find all atoms within 10 Å of the nitrogen atom(s) for each crystal structure. Finally, this coordination sphere was searched to recover the phenyl rings.

To examine the relative position and orientation of the two fragments, each phenyl ring was characterized by three parameters, R, θ and ϕ, as indicated in Fig. 1. R is the distance from the nitrogen atom to the centre of the phenyl ring, θ is the smallest angle between one of the N–C bonds and the vector from the nitrogen atom through the centre of the phenyl ring, and ϕ is the angle between the same vector and the normal to the plane of the phenyl ring.

RESULTS AND DISCUSSION

A superposition plot of the 154 onium groups with all phenyl rings within 8.3 Å of the nitrogen atom is given in Fig. 2a. Only the C atoms of the phenyl rings and the C atoms of the three methyl groups, the first C atom of the R group and the N atom of the onium group are shown. However, after data collection as described above, it was no longer possible to distinguish between a C atom belonging to one of the methyl groups and one belonging to the R group, so that the latter is randomly distributed among the four C atoms of the onium group in the superposition plots. It appears from Fig. 2a that clustering of phenyl rings around the onium group occurs, which indicates an energetically favourable interaction between the two fragments.

The radial density distribution function of the phenyl rings around the onium group is represented in Fig. 3. The relative density of the phenyl rings, $\rho_r(R)$, is the density relative to the density between 7.3 and 8.3 Å. At distances greater than 7.3 Å, the phenyl rings have a random position and orientation, and therefore the density in this area can be used as a reference point. The error

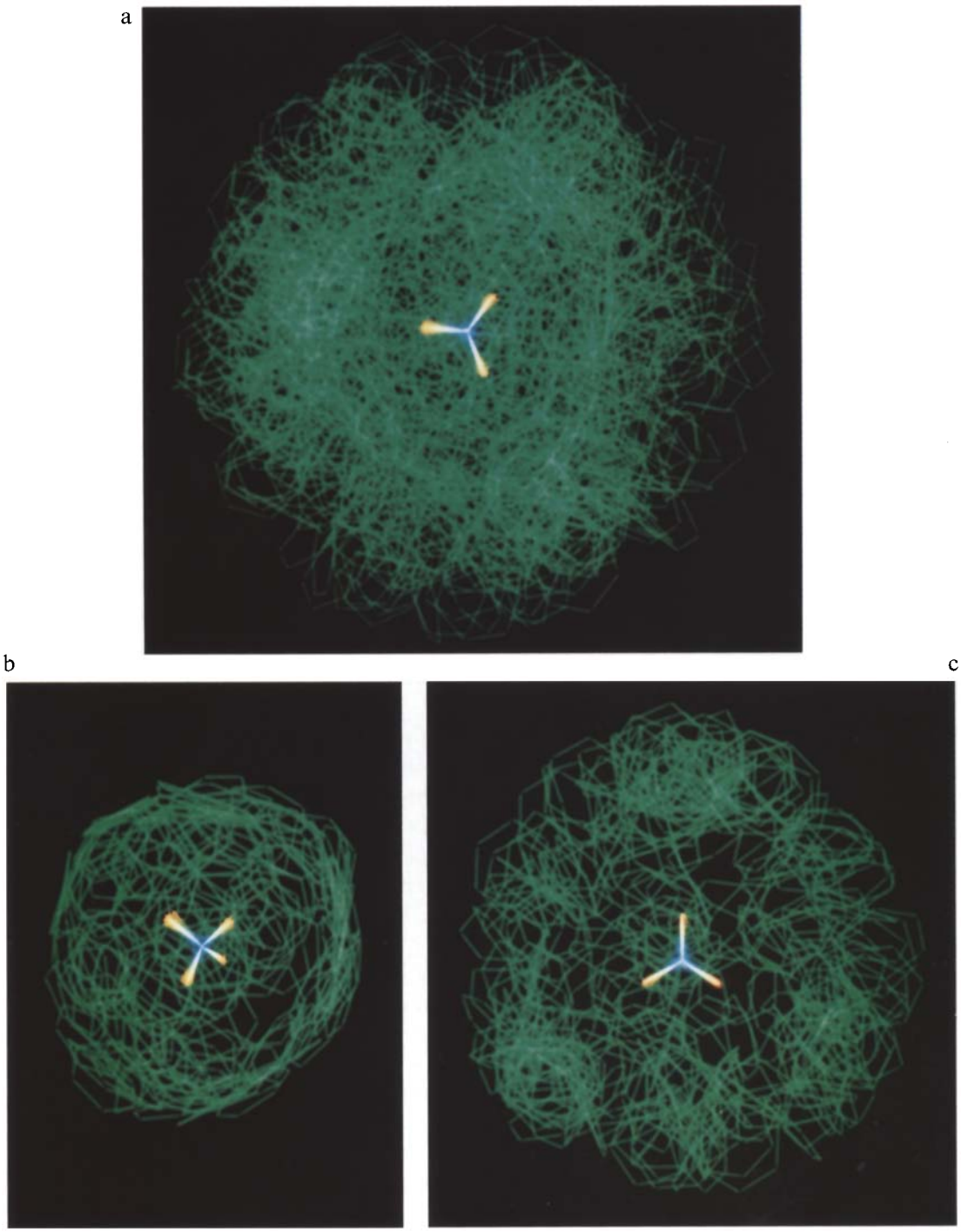

Fig. 2. Plot of the onium groups with the surrounding phenyl rings in which the $R–N^+(CH_3)_3$ fragments are superimposed. (a) All phenyl rings within 8.3 Å of the nitrogen atom; (b) All phenyl rings between 4.0 and 5.0 Å from the nitrogen atom; (c) All phenyl rings between 5.5 and 6.5 Å from the nitrogen atom.

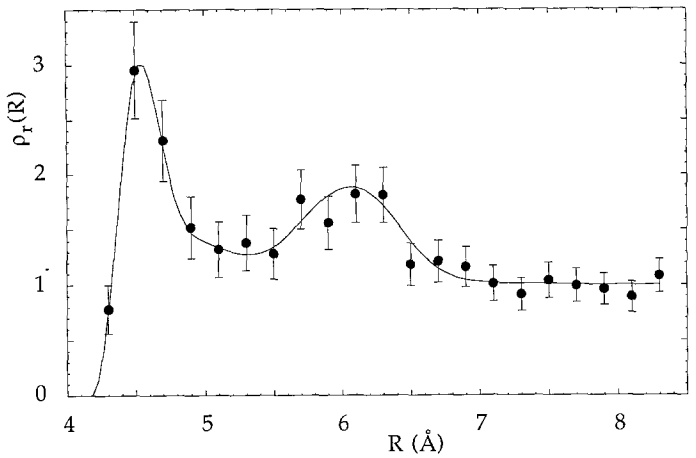

Fig. 3. Radial distribution function of the phenyl rings around the onium group.

bars in Figs. 3, 4 and 5 were calculated assuming a Poisson distribution. There appear to be two maxima in the radial distribution function, at 4.5 and 6.0 Å, respectively.

The density distribution functions of the parameters θ and ϕ are represented in Fig. 4a and Fig. 5a, respectively. $\rho_r(\theta)$, like $\rho_r(R)$, is the density relative to the density between 7.3 and 8.3 Å. However, $\rho(\phi)$ is the number of phenyl rings divided by the geometrical factor, sin ϕ [19]. To see whether the two maxima found in these two density distributions are correlated with those in the radial density distribution function, $\rho_r(\theta)$ and $\rho(\phi)$ were also examined separately for the phenyl rings between 4.0 and 5.0 Å (Figs. 4b and 5b) and for those between 5.5 and 6.5 Å (Figs. 4c and 5c) from the nitrogen atom. There appears to be a correlation, which means that the two maxima in the density distribution functions (Figs. 3, 4a and 5a) correspond to two particular positions and orientations of a phenyl ring with respect to an onium group. These two orientations, which we predict to be energetically favourable, are represented in Fig. 6. In orientation **1**, the phenyl ring is situated in between the substituents (larger θ) at about 4.5 Å from the nitrogen atom, and the ring is approximately oriented on the sphere ($\phi \approx 0$) around the nitrogen atom. The favourable position of the phenyl ring with respect to the substituents cannot be explored in more detail because of insufficient data. In orientation **2**, the phenyl ring is situated in the same direction as one of the N–C bonds ($\theta \approx 0$) at about 6.0 Å from the nitrogen atom and the ring is tilted ($\phi \approx 55°$) with respect to the sphere around the nitrogen atom.

Orientations **1** and **2** can – in a less pronounced manner – also be found in the superposition plots of the phenyl rings between 4.0 and 5.0 Å (Fig. 2b) and of those between 5.5 and 6.5 Å (Fig. 2c) from the nitrogen atom, respectively. From Fig. 2b (orientation **1**) it is clear that the phenyl rings are approximately oriented on the sphere around the nitrogen atom. The position of the rings with respect to the substituents of the onium group, however, is not visible in this plot. In Fig. 2c (orientation **2**), a higher density of phenyl rings is found in the direction of the N–C bonds. The rings at this distance are tilted with respect to the sphere around the nitrogen atom.

It is important to check whether these two orientations are also found in ligand–receptor binding domains. For this purpose we examined the crystal structure of the Fab McPC603–

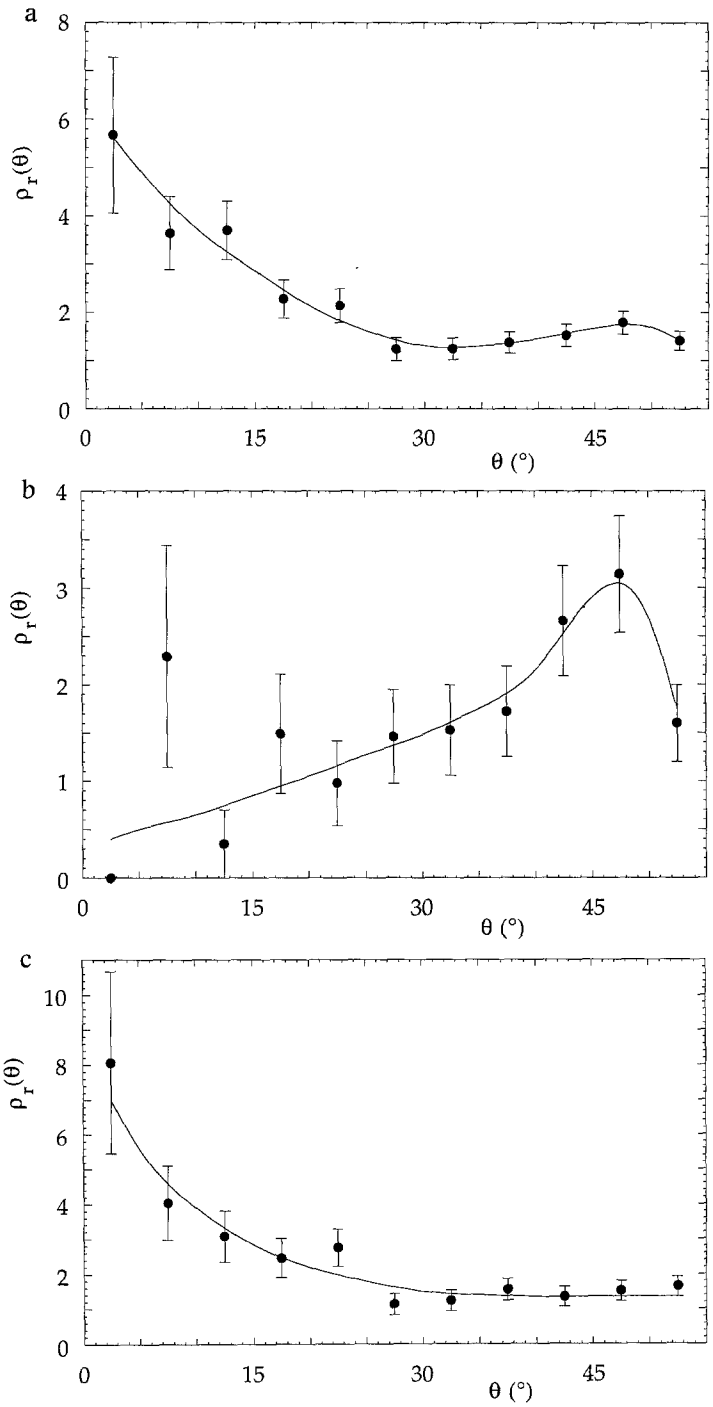

Fig. 4. Density distribution function of the parameter θ. (a) For all phenyl rings within 6.5 Å of the nitrogen atom; (b) For the phenyl rings between 4.0 and 5.0 Å from the nitrogen atom; (c) For the phenyl rings between 5.5 and 6.5 Å from the nitrogen atom.

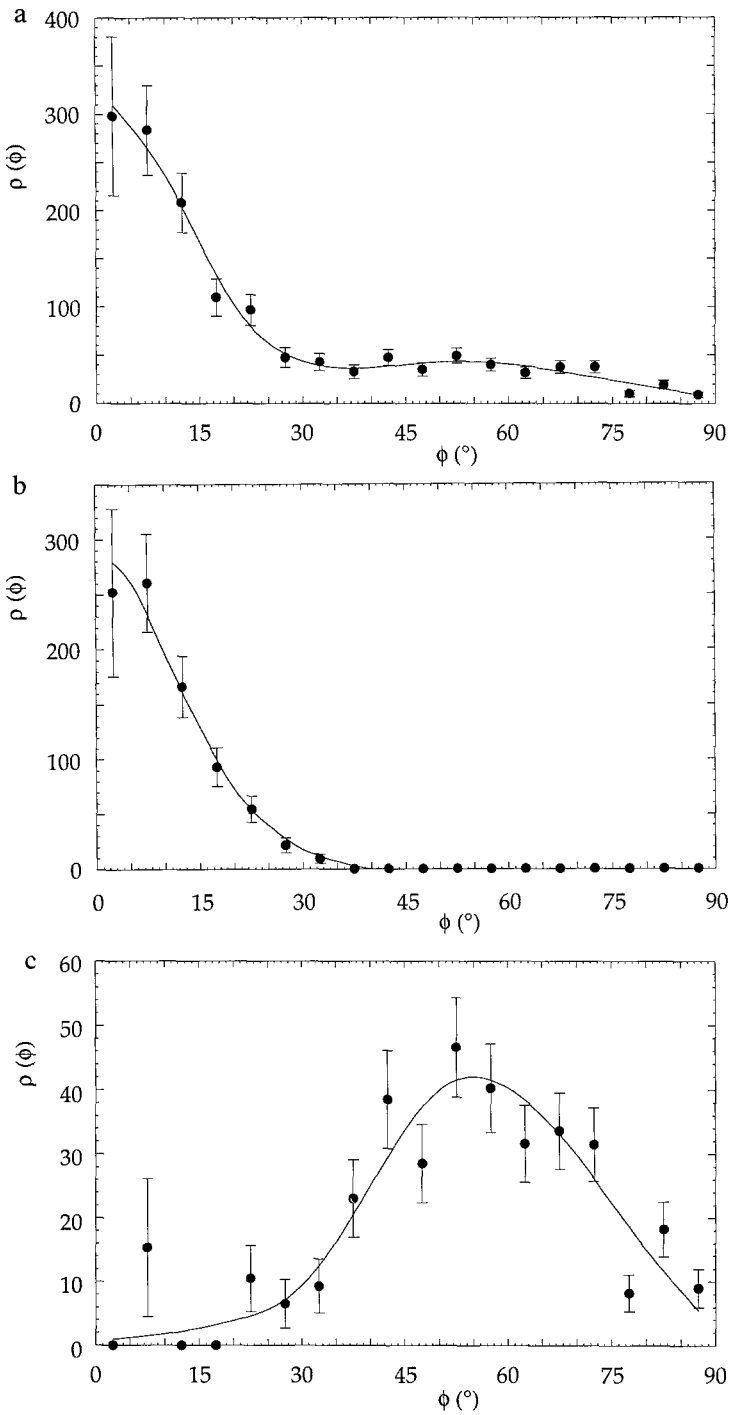

Fig. 5. Density distribution function of the parameter ϕ. (a) For all phenyl rings within 6.5 Å of the nitrogen atom; (b) For the phenyl rings between 4.0 and 5.0 Å from the nitrogen atom; (c) For the phenyl rings between 5.5 and 6.5 Å from the nitrogen atom.

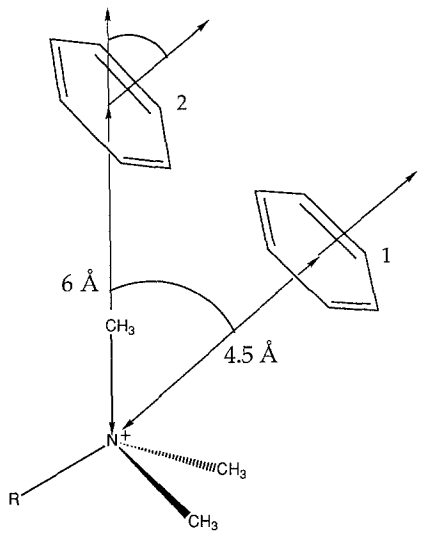

Fig. 6. Representation of the two favourable orientations of a phenyl ring with respect to a $R–N^+(CH_3)_3$ group.

phosphocholine complex (the coordinates were extracted from the PDB; file 2MCP) and two crystal structures of AChE-inhibitor complexes, which were determined recently by Harel et al. (unpublished results). For each of these structures we calculated the parameters R, θ and ϕ for the aromatic rings of the tryptophan, tyrosine and phenylalanine residues surrounding the onium group(s) of the ligand (Table 1). The onium groups of both the AChE inhibitors contain an

TABLE 1
R, θ, ϕ AND THE ORIENTATION OF THE AROMATIC SYSTEMS SURROUNDING THE ONIUM GROUP(S) OF THE LIGAND FOR THREE LIGAND–RECEPTOR COMPLEXES

Residue	R (Å)	θ (°)	ϕ (°)	Orientation
AChE–BW284C51[a] complex				
Phe	6.85	29	54	2
Trp	4.75	56	23	1
Trp	4.64	73	7	1
AChE–edrophonium[b] complex				
Phe	5.44	26	60	2
Trp	4.34	52	16	1
Fab McPC603–phosphocholine complex				
Tyr^{100L}	4.51	38	29	1
Tyr^{33H}	5.54	25	51	2
Trp^{107H}	3.98	44	8	1

[a] Ethyl(3-hydroxyphenyl)dimethylammonium.
[b] 1,5-bis-(4-allyldimethylammoniumphenyl)pentane-3-one.

aromatic substituent which corresponds to the R substituent in our CSD search. One of the other substituents, however, is not a methyl group but an allyl group for BW284C51 and an ethyl group for edrophonium. We defined the direction of these side chains as the vector from the nitrogen atom to the most distant carbon atom of the side chain. The BW284C51 molecule has two onium groups which both interact in the AChE gorge. One interacts with a tryptophan residue and a phenylalanine residue at the bottom of the gorge and the other interacts with a tryptophan residue at the top of the gorge.

In these ligand–receptor complexes, the tryptophan residues were always in orientation **1** with respect to the onium group. The other aromatic rings were in either of the two predicted orientations. The θ values of the rings in orientation **2** were somewhat higher than those found in our CSD search (Fig. 4c), but this is probably caused by the presence of other aromatic rings.

CONCLUSIONS

The fact that we found an increased density of phenyl rings around the $R–N^+(CH_3)_3$ fragment in our CSD search indicates that the interaction between the two fragments is energetically favourable, as has also been found by other investigators [3,20]. The CSD search also provided two energetically favourable orientations of a phenyl ring with respect to the onium group. These findings are reliable because of the experimental character of our data. The same two orientations were also found in the crystal structures of the three ligand–receptor complexes examined. This implies that these orientations probably play a major role in molecular recognition mechanisms. This information could be used to predict the orientation of a ligand at an active site and in this manner be useful in drug design.

ACKNOWLEDGEMENTS

We are very grateful to Michal Harel and Joel Sussman of the Department of Structural Chemistry of the Weizmann Institute (Rehovot, Israel) for providing us with the structural data on the AChE-inhibitor complexes prior to publication.

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