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Review Article

Interactions and Geometry of Aromatic Side Chains in Globular Proteins

Nick Dedes, G. & Stavroula Koulocheri, A.*

Department of Biological Chemistry, Medical School, University of Athens, Greece

***Correspondence to:** Dr. Stavroula Koulocheri, A., Department of Biological Chemistry, Medical School, University of Athens, Greece.

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Abstract

It is known that the traditionally noncovalent interactions like charge-charge interactions, hydrogen bonding, dipolar interactions, London forces and the hydrophobic effect, thought to contribute to the net free energy of stabilization of protein structure and folding, have been appreciated for many years. This is due to their role in protein structure stabilization, ligand -protein interactions and in molecular recognition phenomena. Recently, it has become clear that this is not the whole story. A new class of interactions in proteins involving weakly polar aromatic aminoacid side chains has been observed and characterized. Intermolecular interactions involving arenes are considered specific, with enthalpic contribution, and their importance is defined by their commonality, strength, conservation, and many functions. This review attempts to describe chemically these interactions involving aromatic rings with the respect to protein structure and conformational stability.

Topics addressed are cation-π, arene-arene interactions, hydrogen bonding to π systems, as well as sulfur-aromatic interactions. The geometric pattern and the energetic profile of these weak contacts are also discussed.

Introduction

The unique electronic structure of aromatics involving π electron rich molecules characteristic of the benzene series, gives rise to the interesting and important class of intermolecular forces known as the aromatic interactions. The great interest in aromatic interactions and their role in biological processes stem from their widespread presence. Also, the conservation of aromatic residues in parvalbumins isolated from different species of fish show the importance of preserving these enthalpically favorable contacts of aromatic residues.

This new class of interactions involving weakly polar aromatic aminoacids (Phe, Trp, Tyr) are very widespread throughout nature, as their unique properties make them useful to numerous applications, whose importance is paramount for life. Noncovalent interactions involving aromatic rings are of great importance for proteins especially for structure stability, folding, protein-protein recognition, ligand binding and consequently drug design. DNA and RNA bases are aromatic, and the stabilization of their helical structures depends on base stacking. In addition aromatic residues in proteins participate to DNA and RNA recognition.

This kind of interaction is conducted through an aromatic residue and various molecules including other aromatic compounds or other groups interacting with π systems. The formation of aromatic dimers and even aromatic clusters of more than two aromatic residues, has essential implications on protein function, stability, and ligand recognition [1]. Specifically, the molecular structure in Alzheimer's amyloid proteins, as well as the vast majority of medicinal agents, such as anti-Alzheimer drug E2020 (Aricept) [2] (Fig.1)[3] and many anticancer pharmaceuticals, contain aromatic substituents and their differential recognition by proteins is dominated by diverse π interactions.

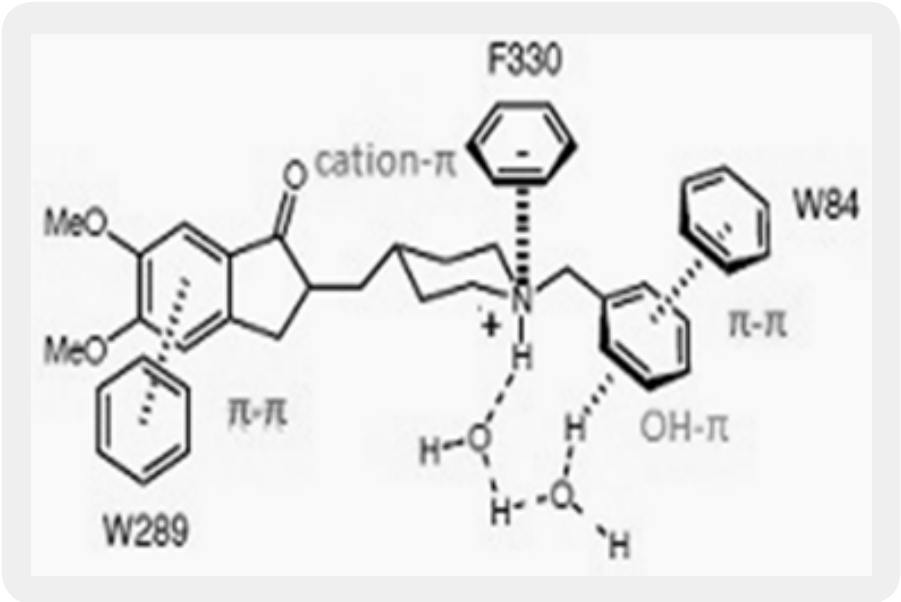

Figure 1: *Binding mode of the anti-Alzheimer drug E2020 within the active site of acetylcholinesterase from* ***Torbedo californica*** *[3]. Meyer EA, Castellano RK, Diederich F. Interactions with Aromatic Rings in Chemical and Biological Recognition. Angew. Chem. Int. Ed. 2003; 42: 1210 – 1250. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.*

Their strength, physical nature and specificity vary drastically. Exceptionally worthy of attention is however, the role of those interactions in globular proteins where their effect is influencing the tertiary to a great extend (80%) and less the quaternary (20%) configurations and therefore determine the functionality of the protein. Moreover one intriguing discovery by Burley and Petsko suggests that 60% of the aromatic side chains participate in aromatic pairs, 80% of which form networks of three or more interacting aromatic side chains [4,5].

The contribution of the aromatic interaction to the overall stability of the protein can be easily comprehended if the thermophylic proteins are taken into account, in which the extensive presence of such contacts protects them falling apart in the arduous native environment of the organisms who produce them [1]. Even more striking is the fact the aromatic rings tend to array in two fixed ways in proteins and at a preferred angle and distance between them. Finally, these arrangements are the most energetically favourable and justify the great contribution of these conformations to the stability of the protein [4].

This review attempts to present a general background on these aromatic interactions involving the different partners to π systems, providing their geometric pattern and their energetic insight for a better understanding of this biologically important noncovalent interaction.

Aromatic Interactions

Because these interactions vary in their nature, partners, strength and specificity, it is better to describe them individually.

Cation-π

It is known that the "non classical" A–H...π hydrogen bonds can shift to a region of a different kind of interaction much stronger, the so called cation…π interaction. There are complexes where the group X-H is a cation like $-NH_3^+$ (interaction energy about -19 kcal/mol). The binding energy is dominated by purely electrostatic energy of the positive centre and the π electron cloud. This means that the N^+-H …π interaction lies in between a cation..π interaction (like K^+…π) and a pure aromatic hydrogen bond of the type X-H…π. In proteins, cation…π interactions are favorable interactions between positively charged groups (Arg or Lys) or groups with partial positive charge δ^+ on their group and π-aromatic systems. These non-covalent interactions are also important to stability and contribute to the specificity of binding. Experiments have convincingly shown the stabilizing role that such contacts can make within proteins and at their surface.

Cation …π interactions are now as important as hydrogen bonding and hydrophobic effects in protein structures [6]. The cation…π interaction is a noncovalent molecular interaction between the face of an electron-rich π system (e.g. benzene) and an adjacent cation. Cation …π effects, with the assumption of the "electrostatic model" of the cation…π interaction [7], are important contributors to the structure and function of biomolecules. It seems that this interaction might likely be a powerful organizational force in protein structure [8].

Cation…π interactions play an important role in nature, particularly in protein structure, molecular recognition and enzyme catalysis. The effect has also been observed and put to use in synthetic systems. The model π system, has no permanent dipole moment, as the contributions of the weakly polar carbon-hydrogen bonds cancel due to molecular symmetry [9,10].

However, the electron-rich π system above and below the benzene ring hosts a partial negative charge. A counterbalancing positive charge is associated with the plane of the benzene atoms, resulting in an electric quadrupole (a pair of dipoles, aligned so there is no net molecular dipole moment) (Fig. 2) [11]. It is topologically analogous to the dz^2 orbital. It is known that sp^2 carbons are more electronegative than a proton. This gives rise to six dipoles that form the molecular quadropole of benzene[11]. This interaction is a noncovalent bonding between a monopole (cation) and a quadrupole (π system). The electrostatic interaction and the interactive geometry arise from the cation interacting with arene quadropole moment [12,13].

The negatively charged region of the quadrupole can then interact favorably with positively charged species; a particularly strong effect is observed with cations of high charge density.

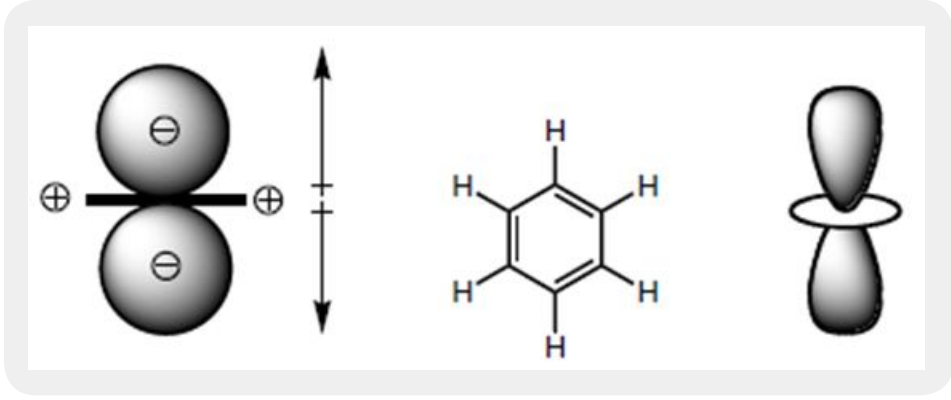

Figure 2: *The creation of six $C^{\delta-}$ -$H^{\delta+}$ bond dipoles (an sp^2 C is more electronegative than H) and under the symmetry of benzene, they give rise to a molecular quadrupole [11]. R. Knowles. Aromatic Interactions. http://www.princeton.edu/chemistry/macmillan/group-meetings/cation%20pi.pdf Reproduced with permission.*

Cation…π interactions in water have been investigated by Dougherty [14] studying cation …π energies for the alkali metals in aqueous solution. Many cation… π interactions in proteins are located at the surface, there salt effects are very weak. This shows the importance of cation-π contacts in protein structure [15]. Electrostatics and cation-induced polarization expain the various binding energies in alkali-metal…π interactions [16,17].

The very important review of Ma and Dougherty[7] determines the essential features of the cation…π interaction concerning its structure, energetics and biological implications. Further studies emphasize its importance to structural biology, for the stabilization (within and at their surface) of protein structures [18], contribution to ligand structure and catalysis [19], and applications in host-guest chemistry [3]. Cation…π interactions between an aromatic side chain and the ammonium group on lysine (Lys) or the guanidinium group on arginine (Arg) are known in protein structures [18].

Amino…arene interaction between positively N-H groups and aromatic amino acid side chains has been found in proteins [20]. In this case, the positive charge on the cation is attracted to the partial negative charge on the face of the aromatic ring. These interactions are characterized by a specific geometry, which means *"directionality"*. Interestingly, the geometry with the greater frequency for the interaction of Lys with the aromatic π-system is the packing of the ε-CH_2 of the Lys side chain into the face of the aromatic ring [18,21].

This poses the question as to whether this can still be considered to be a cation…π interaction or there is a preference for a hydrophobic interaction.

Marcey L. Waters [22] found that in the context of a β-hairpin peptide, Lys interacts with Trp most often via its ε-CH_2 group, and not directly via the ammonium group, because the ε-CH_2 takes a partial positive charge for the reason that NH_3^+ has a negative inductive effect. This supplies a polar yet hydrophobic group. This fits perfectly with the polar hydrophobic face of the aromatic ring, and a specific interaction occurs at that position in the chain. This address the question of whether this ε-CH_2…π interaction has cation-π character or it is simply a (nonspecific) hydrophobic interaction. The ε-CH_2, adjacent to the aromatic ring, presents the place for greatest interaction. Consequently, the NH_3^+ group interacts indirectly with Trp, turning away from the aromatic ring to benefit from solvation by water.

Interestingly, it has been shown that an Arg-Trp interaction occurs via stacking of the guanidinium group with the Trp, and that the interaction is more favorable than the Lys-Trp interaction mentioned above [22,23].

π–π Interactions

Biological Importance

It is known that the implications of π–π interactions in chemistry and biology are broad and of great importance. This class of weak interaction involves direct attraction between arene rings.

An aromatic ring is not non-polar, it has a quadrupole moment as mentioned before, which gives rise to a fixed non uniform charge surface distribution. Because an aromatic ring is a hydrocarbon, it is hydrophobic but not as hydrophobic as the aliphatic group. The combination of these characteristics of the aromatic ring results in unique properties in its noncovalent interactions, quadrupole-quadrupole interactions. These interactions have orientation preferences of the involving aromatics rings. This important characteristic, the fact that these interactions occur in a specific geometry, gives a moderate *directional character* like hydrogen bond, conferring specificity [22]. The weakly polar feature of the aromatic residues suggests that Phe, Trp and Tyr should be able to conduct interactions between themselves. The π-π interactions refer to attractive, noncovalent interactions between aromatic rings with the contribution of the van der Waals contacts. In fact, the major contributions to the interaction energy come from the van der Waaals and electrostatic components, while the dominant electrostatic interaction has to do with the association energy of the complex.

These interactions are important in nucleobase stacking within DNA and RNA molecules, protein folding, template-directed synthesis, and molecular recognition. Burley and Petsko [4,24], have shown that a lot of π-π interactions between aromatic amino acid side chains exist in proteins. Also, it is known that π-π interactions are involving in folding [25] and in the thermal stability of proteins [26]. Edge-to-face geometry interactions which will be discussed in the next paragraph, exert an effect on pka of Tyr, increasing –OH acidity.

Stacking π-π interactions are essential in the organization of porphyrins inside proteins [27] and play key role in the self-assembly of amyloid fibrils [28]. Moreover, the Phe–Phe interaction can stabilize monomeric α helices by up to ΔG=-0.8 $kcalmol^{-1}$, as determined recently by circular dichroism studies [29].

Geometry and Energetics

In 1990, Hunter and Sanders [30] in their pioneering work began to investigate and analyze in detail this class of interactions. According to their electrostatic model, for a qualitative approach to the understanding of π–π interactions, the σ and π systems are considered separately, there is interaction between arene molecules when π-σ interaction is stronger than π-π repulsion which destabilize the complex.

There are regions where favorable or attractive interactions and unfavorable or repulsions between arene rings occur. Analyses show that the two aromatic rings tend to be positioned in very specific and enthalpicaly favourable geometric arrangements. The interactions of aromatic rings are a function of orientation angle and distance [31]. This remarckable finding, excludes the possibility of random close packing of planar molecules in the interior of a protein.

Application of this mentioned electrostatic model, gives a set of rules to a better understanding of π-π complexes

1. π-π repulsion terms disfavor the face to face interaction
2. π-σ attraction terms dominate the edge to face interaction
3. π-σ attraction terms dominate the offset (or parallel displaced) interaction

Substituent effects become very important in ordering the balance between attractive and repulsive forces [11].

The benzene dimer has been used as a model system for the study of π–π interactions. There are three proposed configurations of the benzene dimer with the lowest energy: Edge to Face (T-shaped), Parallel Displaced (offset) and Face to Face (Fig. 3).

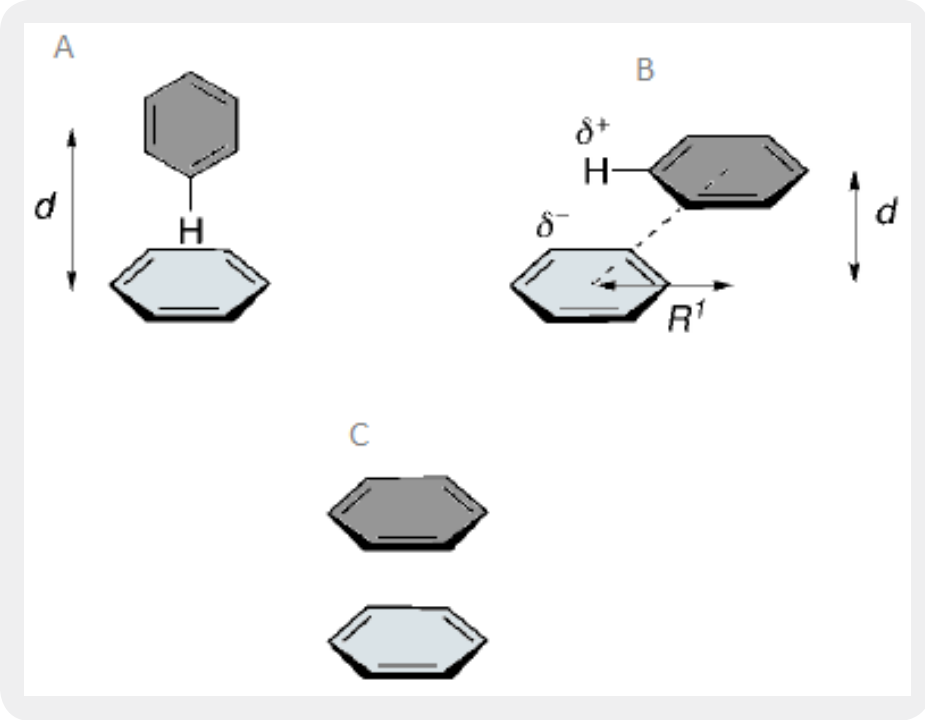

Figure 3: *Proposed lowest energy structures of the benzene dimer. d: distance between plane rings, R1 lateral offset. A. Edge to Face (T-shaped) B. Parallel Displaced (offset) C. Face to Face [3]. Meyer EA, Castellano RK, Diederich F. Interactions with Aromatic Rings in Chemical and Biological Recognition. Angew. Chem. Int. Ed. 2003; 42: 1210 – 1250. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.*

It is known that London dispersion interactions are the major source of stabilization energy between two aromatic molecules; despite this, benzene has a great quadrupole moment and this directional electrostatic contribution is great in determining the geometry of the interaction.

For example, the parallel-displaced configuration is an adjustment between optimal surface overlap with the maximum of the dispersive attraction (distance dependence r^{-6}) and quadrupole moment positioning (dependence r^{-5}). Also short-range repulsive interactions exist which modify the interplanar distance [3].

Two nearly isoenergetic minima have been reported for the edge-to-face and parallel-displaced arrangements. The parallel-parallel configuration C (Fig. 3) is considered more unfavorable than the other two. The three configurations are in dynamic equilibrium and the energy barriers for interconversion are very low [32]. It is necessary to mention the geometric parameters corresponding to the most stable configurations A and B. Configuration A has ring-center separation of 4.96 Å with the partially positively charged H atom pointing perpendicular into the partially negatively charged center of the second ring (Fig. 3). The parallel-displaced stacking dimer B has an interplanar distance d of 3.4-3.6 Å, with a displacement R^1=1.6-1.8 Å.

Despite of a HOMO-LUMO charge-transfer interaction (about 0.7-1.5 $kcalmol^{-1}$) in parallel-parallel configuration C which is less stable than T-shaped configuration [33], this arrangement is unfavorable because of repulsions between electron clouds or quadrupole moments of equal sign [3].

It is assesed that in proteins pairs (dimers) of aromatic amino acids side chains, there is a preferential alignment of their respective aromatic rings in an off-centered parallel orientation and not in a parallel, centroid to centroid arrangement. Also, the parallel-displaced conformation contributes by 1.5 kcal/mol to the stabilization of the molecule, while the contribution of the parallel centre to centre formation is almost non-existant. In addition, aromatic dimers have also been observed to adopt a T-shaped arrangment. It has been observed that the parallel-displaced structure is 0.5-0.75 kcal/mol more stable than the T-shaped one. It should be mentioned that in T-shaped interactions, besides the substantial dispersion component of the interaction, the electrostatic directional contribution to this interaction depends on the *"acidity"* of the interacting edge H atom, which means how partial positive charge exists on H. In the case of aromatic amino acids (Phe, Trp, Tyr) side chains, strong electron withdrawing groups/substituents does not exist, thus there is not enhancement of $H^{\delta+}$ positive charge, which means that the electrostatic factor is a less effective factor to this interaction. In terms of acid-base chemistry, this interaction becomes stronger increasing acidity of H or increasing the basicity (increasing π electron density) with electron-donating groups of the other interacting π system.

Besides the T-shaped interaction, the parallel arrangement must be mentioned. This complex formation needs one electron deficient aromatic ring (bearing strong electron-acceptor groups) and one other electron rich partner (bearing strong electron-donor groups). Molecular orbital interactions determine the geometry of the complex (HOMO–LUMO charge-transfer interaction). These stacking interactions can be explained by the electrostatic model of Hunter and Sanders [30]. The concept "polar/π" interaction shows the significance of polar, electrostatic term in π-π stacking. It is possible to explain better stacking interactions by examining the quadrupole moments of the arene partners, or observing the molecular electrostatic potentials (MEPs) [3].

A common misconception is that aromatic groups should stack on top of one another in a face-to-face arrangement. Only in cases where there is complementarity of charges or polarizations between the aromatic partners, thus attractive electrostatic interactions can occur; in general, π-electron repulsion disfavours the stacked arrangement [30].

Even in the nucleic acids where "stacking" is usually referred as stabilizing factor of the double helical structure, true face-to-face stacking with extensive π-overlap rarely occurs, more often the bases adopt a favorite offset or twisted conguration instead of this face-to-face configuration [34]. There is a fundamental difference in the case of mentioned "π-π parallel stacking" interactions made by the consecutive bases in the interior of the DNA double helix. This difference can be attributed to hydrogen bonds formed between the opposite pairs and because 50% of the bases are heterocyclic. Finally, supramolecular configurations like helices, have been constructed through parallel stacking interactions.

X-H… π Interaction

The question as to whether the X-H…π interactions can be considered as hydrogen bonding, may be replied in the affirmative. Despite the fact that X-H…π hydrogen bonds are not well studied in biology, they have been recognized for many years. A more detail understanding and knowledge of the X-H …π interactions can be achieved by crystallographic techniques.

The CH…π Interaction

It is known that hydrogen bond has been defined as A-H···B, where the donor A and acceptor B are electronegative atoms. This concept of "classical" hydrogen bond formulated by Pauling has been extended and revised to a "non classical" type of hydrogen bond/interaction, more generalized with unusual donors and acceptors like carbon and π-systems (CH…π). The large face of the aromatic ring can serve as acceptor, including the C atoms. This makes the aromatic acceptor an easy target and with flexible directionality for such interactions with hydrogen bond donors occurring even in adverse stereochemical environments. In crystal structures aromatic acceptors are used to compensate the lack of local strong hydrogen bonding acceptors. If there are many donors, they can satisfy the hydrogen bonding potential contacting the weak aromatic acceptors. Typical examples are observed in proteins where hydrogen bonding donor amino groups contact aromatic acceptors. The CH…π hydrogen bond, a weak molecular force occurring between a soft acid C-H and a soft base π- electron system, is one of the most significant and plays a functional role in terms of conformation and stability of 3D macromolecular structures (proteins, nucleic acids, lipids and polysaccharides), as well as in many molecular recognition events [35,36].

For weakly acidic C-H donors for uncharged molecules, C-H…π interactions fall into the region intermediate between the hydrogen bonds and van der Waals interactions.

The CH…π interaction makes an important contribution to weak intermolecular forces and is of great value to chemical, and biochemical sciences. The implication of this interaction is great in defining the conformation of biological macromolecules.

Though these interactions are individually quite weak, their effects can be additive in macromolecules with noticeable effects, a salient feature is that the CH…π hydrogen bond can work cooperatively. It should be pointed out that it works in nonpolar as well as polar, protic solvents such as water. A great contribution comes from the dispersion energy in cases where aliphatic or aromatic CH groups are involved. Coulombic energy is of minor importance as compared to the other weak hydrogen bonds. The hydrogen bonding nature of this interaction, however, has been confirmed by AIM (atom in molecules technique) analyses [37]. The nature of the CH… π interaction arises mainly from dispersion and partly from charge-transfer (π-σ*) and electrostatic forces. This interaction is a weak (1.5-2.5 kcal/mol) hydrogen-bonding type, orientation dependent and directional from the electrostatic contribution. Also the strength of the interaction depends on the nature of the molecular residues, CH and π-system: the stronger the proton donating ability or acidity of the CH group, the larger the stabilizing effect. The hybridization state of C and inductive effects by nearby groups exert an effect on interaction energy. When C-H acidity is decreased, the hydrogen bond nature of this interaction declines and blurs into the van der Waals region.

It should be mentioned that the well-known edge-to- face π-π interaction which has definitely a directional characteristic and often named as "herringbone interaction", (it is a driving organizational force in crystal structures) is differenciated from a hydrogen bond. The field of the CH…π interactions is comprehensively studied by Nishio *et al.* [38]. Nichio calls all these contacts CH…π interactions. Generally, there is not a well defined border between the herringbone interactions and hydrogen bond and in some cases is difficult to classify these contacts precisely [36].

O-H… π, N-H…π Hydrogen Bonding to Aromatic π Systems

The energetically favorable contacts between hydrogen bond donors and aromatic rings (the ring conceptually similar to a very large flat weak base) are called π-hydrogen bonds. They are weaker than classical H bonds. Alcohols, amines and amides can bind to aromatic rings (Fig. 4).

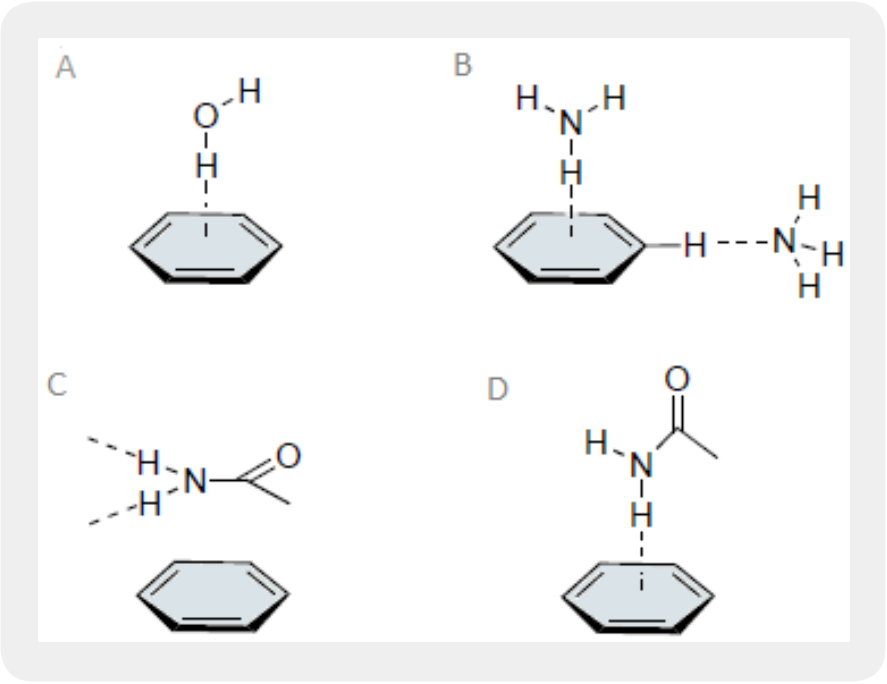

Figure 4: *Preferred interactions of benzene with water (A),ammonia (B), and amides (C,D) [3]. Meyer EA, Castellano RK, Diederich F. Interactions with Aromatic Rings in Chemical and Biological Recognition. Angew. Chem. Int. Ed. 2003; 42: 1210 – 1250. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.*

It is obvious that the strength of OH…π hydrogen bond depends on substituents (with positive or negative inductive effects) on the acceptor. In the complex of benzene…H_2O, oxygen is above the center of the aromatic ring and hydrogen points to the π cloud (bidendate geometry).

The O-H…π contacts are mainly between proteins and water and concern protein hydration. There are also interactions of water molecules with the aromatic rings of Phe, Trp, and Tyr inside hydrophobic cavities providing further stabilization locally in protein configuration and may make significant contributions to stabilizing the local protein structure [39].

On the other hand, statistical analyses of protein structures show that water molecules interact with the edges of aromatic rings of the type C-H···O with the δ^+ polarized hydrogen atoms [40]. Despite the fact that hydrogen bonds of the general type X-H…π are unusual in biology, they have been recognized for many years.

Given the fact that each amino acid has the possibility to act as a N-H hydrogen-bond donor as well as the side chains of Lys, Arg, Asn, Gln, His, there are many possible N-H…π contacts in proteins.

A N-H…π interaction was seen by Perutz *et al.* in the X-ray crystallographic study of the interaction of hemoglobin with the drug bezafibrate, an asparagine side chain N-H residue points into the center of the p-chlorobenzamide ring of the drug [41]. Also there are many other examples of N-H…π, O-H…π and S-H…π contacts that have been observed in proteins from crystallographic studies. The effect of N-H…π interaction on the secondary structure of proteins has been studied [42].

The presence of strong hydrogen bonding donors contribute to an interaction energy in the range of -2 to -4 kcal/mol. The order of hydrogen bonds donor strengths is O-H>N-H>C-H.

Contacts between N-H donors and aromatic side chains are not observed very often in proteins. An aromatic ring can be considered as "reserve" acceptor for a strong donor. Tyr, Phe, and in particular Trp side chains interact more frequently with polarized CH groups as donors [43].

Despite the fact that N-H… π interaction is considered stronger than the C-H… π interaction, the energy gain is overcompensated by a greater desolvation cost, because a strong donor will make better interactions with a stronger acceptor than with an aromatic ring [44].

In case of a sp^2 –hybridized nitrogen atom in a N-H hydrogen donor, because this geometry of nitrogen is trigonal planar, a parallel stacking occurs instead of the T-shaped configuration [45]. Generally, intermolecular X-H…π hydrogen bonds occur with widely differing geometry in crystal structures. The centered geometry occurs occasionally as ideal geometry. More often, the hydrogen bonding geometries deviate from perpendicularity. Also, donor and acceptor directionalities are important in the context of crystal structure.

Crystallographic evidence shows that 1 out of 11 aromatic amino acids acts as a π acceptor for H bonding with O-H, N-H, and S-H donors, with Trp acting as the best acceptor. This noticeable frequency, although small compared to usual hydrogen bonds, emphasizes the important role of these interactions in the structure and stabilization of proteins [43,3].

In crystals, interaction of ammonium groups with phenyl rings are clearly directional and posses the IR spectroscopic features of hydrogen bonds [46]. The interaction energy is dominated by strongly electrostatic interaction of the positive centre and the π phenyl electron cloud. This shows that this interaction lies in between a cation…π interaction and a pure aromatic contact. It is noticeable that in protein crystals formation does not dominate hierarchy based on strong donors and acceptors. In protein crystals the packing is determined by the cooperation and competition between a large number of strong, weak and very weak interactions [36].

Finally the theoreticians have not reached a *consensus* on the contribution of dispersion, charge -transfer, electrostatic and repulsion phenomena to the different kinds of hydrogen bonding.

Hydrogen bond is an interaction with widespead borders-on the weak side there is a gradual shift to the van der Waals continuity and on the strong side there are also gradual shifts to the covalent bond, to the purely ionic electrostatic interaction and also to the less analyzed completely but strong cation…π interaction.

Amide-π Interactions

Hydrogen-bonding contacts between the N-H of amide groups and the side chains of aromatic amino acids play a significant role to protein stability and to protein–ligand binding. The N-H···π interactions between amides and aromatic amino acids have been studied with peptide model systems as in the case of inhibitors binding to Chk1 kinase where there are N-H···π interactions between the aromatic ring of the inhibitor and the amide NH (of Ser147/Asp148) of kinase [47].

Concerning amide-π interactions in protein structures, the amide often stacks over the surface of the arene. There is evidence from protein crystal structures that acylated Lys forms amide- π interactions in the context of protein-protein interactions and may play a role in protein recognition events. Stacking of the amide group with the a Trp ring, results in a free NH group to hydrogen bond with water, like a stacking of Arg with Trp. This suggests that a Trp-AcLys interaction may compensate for the loss of the cation-π interaction (charge-quadrupole) making a π-π interaction component and additionally a dipole-quadrupole interaction between the amide bond and the aromatic ring [22].

Sulfur…Arene Interactions

Understanding the S···Ar interaction in proteins, in enzyme catalysis (enzyme-substrate complexes) has important consequences, regarding stability, binding and geometry. Besides the contribution to the understanding of proteins, there is also a major contribution to modern drug development. There is convincing evidence for the stabilizing role of S…arene interactions (especially Smet-arene). From mutation experiments, a protein destabilization occurs upon mutation of a Met residue (to Leu) close to Phe and Tyr side chains [48]. The nature of sulfur…arene interactions in protein environments has been investigated and reviewed [3,47].

With the analysis of the continually increasing number of protein crystal structures, S···Ar contacts are studied in a more efficient way in terms of geometry and energy models. Close contacts between sulfur-containing amino acid side chains (Met, Cys) and aromatic side chains have been observed in globular proteins from crystallography. The conserved disulfide…tryptophan interaction in immunoglobin (Ig) proteins (PDB code:1IEA)[49] shows the importance of this type of contact.

Methionine … aryl interactions exist very often in protein structures mainly in the environment of the tryptophan indole ring [50].

It should be useful to mention the atomic characteristics of sulfur for a better understanding of this type of interaction. Chemically it is known that sulfur is characterized by: filled 3p and empty 3d orbitals, great polarizability compared to carbon (sulfur is a larger atom so it has more, loosely held electrons than carbon) and the acidity of S-H group of cysteine residues (with the possibility of interaction with the π surfaces).

Also divalent sulfur containing molecules (particularly disulfides) have the ability to quench the fluorescence of aromatic amino acids [3]. Divalent sulfur can interact both with electron-poor and with electron-rich chemical groups. The electron-rich ones tend to be directed towards the antibonding s* orbitals of the S-C (σ* direction), while electron-poor ones (the partially positively charged (δ^+) edge of the arene) orient towards the sulfur lone electron pairs [51-53].

From PDB studies, Met side chains are often close to a π donor-arene and amides[54] Also very frequent C-H···S contacts are observed, which suggests dominant dispersive interactions with Met. Cys side chains show preference for S-H···π hydrogen-bonding type interactions over dispersion interactions of the type C-H···S [47].

Generally, with regard to phenyl ring interactions, due to the great sulfur polarizability, divalent sulfur acts like a weakly negatively polarized atom.

Fig. 5. shows the probable interaction geometries for the side chains of Met (Fig.5A) and Cys (Fig.5B) with aromatic rings [47].

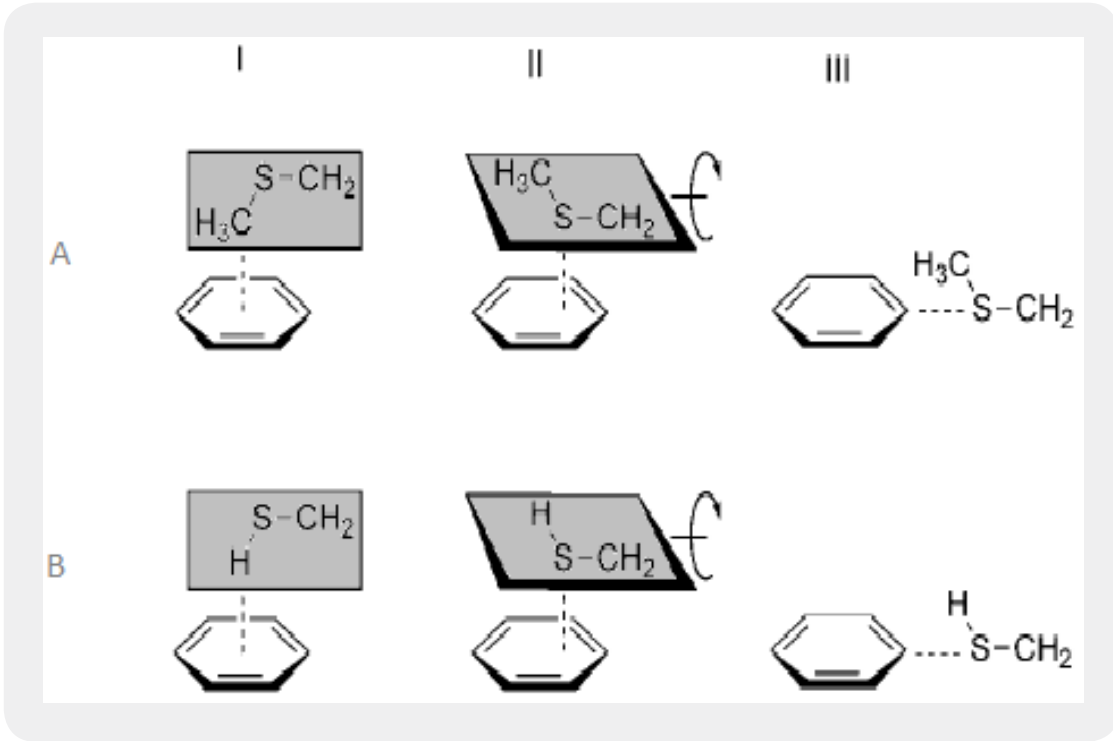

Figure 5: *Geometries for Met-arene A. and Cys-arene B. interactions in proteins: The arrows indicate the variation of the angles between the aromatic ring plane and the planes through the Me-S-CH_2 and H-SCH_2 fragments [47]. L.M. Salonen, M. Ellermann, F. Diederich, Aromatic Rings in Chemical and Biological Recognition:Energetics and Structures, Angew. Chem. Int. Ed. 2011; 50: 4808-4842. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.*

For Met side chains, the geometry is above the face (Fig. 5A I,II), as well as at the edge of the ring. (Fig. 5A.III) [50,55].

For, the side chain of Cys, there is a contact mode S-H···π (Fig. 5B.I) [56]. Also from PDB studies there is the geometry type II as shown in Fig. 5B.II [50], giving a possibility to Cys for participating in hydrogen bonding (of the type S-H…O or N) as a common hydrogen-bond donor to other hydrogen bond acceptor like O or N in proteins. The geometry Fig. 5B.III has also been observed in proteins. In all the geometries, the sulfur displacement from the center of the arene has angular preferences 30°–90°), so more favorable interactions can occur with the aryl ring [52,53].

The dominant force in sulfur…arene interactions is the dispersion force and secondarily the electrostatic force which may affect the geometry of the dimer.

Generally, the concept of sulfur–arene interaction can involve various interaction contacts like: S···π, S-H···π, S-C-H···π, and C-H···S [57,47].

Conclusions-Future Research

This review attempts to present a general background on these biologically important weak noncovalent aromatic interactions in terms of their driving force, strength, structure and contribution to the overall structure and conformational stability of the protein structure. It is known that these weaker interactions are less well-defined and the general picture is not so clear. The contribution of the totality of aromatic contacts with the rest of the protein into the overall stability of a protein can explain the conservation in evolutionarily related proteins.

The progress that has been made the last years in research concerning the features of these individual intermolecular bonding interactions in terms of structure and energetics, has enhanced our knowledge in this field. The analysis of the increasing number of protein crystal structures by computational tools and the experimental findings from quantitative studies, biostructural studies, crystallographic and protein database mining in CDB and PDB, have provide insight into this type of π intermolecular interactions in proteins and in molecular recognition phenomena. There is indication that random close packing of nonpolar groups cannot dictate completely the internal geometry of globular proteins and aromatic rings can interact in a highly nonrandom fashion. So it is not possible that geometric arrangement could arise as the result of a random orientation.

From studies, it is certain that there is a gain with respect to the free enthalpy from interactions with individual arene rings, and the gain is greater with increasing number of aromatic rings. The geometry and energetics of π interactions involving T-shaped, parallel displaced and face to face are investigated actively for further optimization in geometry and energy profile. Recent work has demonstrated that aromatic interactions can provide selectivity as well. Also implications of aromatic-aromatic interactions[58] are of special interest in biomedical research because of applications in modern drug design, next generation drugs and drug delivery systems [59].

The sulfur-arene interactions need further investigation and study. Given the fact that hydrogen bonding is an interaction with widespread borders, H bonding to π rings is poorly analyzed. This field of π interactions is very attractive in future research, and further experimental studies, computational analyses and quantifications of π interactions in model systems are needed. Applications of aromatic interactions for diverse processes are continuously growing [60]. Apart from π-π dimers, aromatic residues participate to the formation of π-π networks of aromatic clusters in various conformations [61]. Also aromatic clusters need further in depth study and research. A thorough knowledge of this class of weaker noncovalent interactions is crucial to the understanding of biological complexity in proteins.

It seems that more biological examples involving π interactions in proteins will appear in the near future.

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