Rediscovery of Halogen Bonds in Protein-Ligand Complexes

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Abstract: Although the halogen bond has attracted much interest in chemistry and material science communities, its implications for drug design are just now coming to light. The protein–ligand interactions through short halogen–oxygen/nitrogen/sulfur contacts have been observed in crystal structures for a long time, but only in recent years, with the experimental and theoretical progress in weak biological interactions, especially the pioneering works contributed by Ho and co-workers (Auffinger, P.; Hays, F. A.; Westhof, E.; Ho, P. S. *Proc. Natl. Acad. Sci. USA* 2004, *101*, 16789–16794), these short contacts involving halogens in biomolecules were rediscovered and re-recognized as halogen bonds to stress their shared similarities with hydrogen bonds in strength and directionality. Crystal structure determinations of protein complexes with halogenated ligands preliminarily unveiled the functionality of halogen bonds in protein–ligand recognition. Database surveys further revealed a considerable number of short halogen–oxygen contacts between proteins and halogenated ligands. Theoretical calculations on model and real systems eventually gave a quantitative pronouncement for the substantial contribution of halogen bonds to ligand binding. All of these works forebode that the halogen bond can be exploited as a new and versatile tool for rational drug design and bio-crystal engineering.

Keywords: Halogen bond, σ-hole, protein-ligand interaction, rational drug design.

INTRODUCTION

Noncovalent interactions that are indicative of attractive intermolecular forces have always been of key interest to pharmacologists in their search for the "glue" that specifically holds drugs and their cognate targets together. One of the well known chemical forces is the hydrogen bond which has been widely recognized in the chemistry and biology communities [1]. Short contacts between the covalently bonded halogens and the lone pair-possessing atoms (such as O, N and S) were observed as early as two centuries ago [2,3] and termed as charge transfer bond by Hassel in his Nobel lecture in 1970 [4]. Since then, the ability of this interaction form (also referred as halogen bond today to stress its similarity to hydrogen bond [5–8]) to function as general, effective, and reliable sites for directing molecular recognition processes was remained largely underappreciated until the 1990s. During the past decade, halogen bond was exploited to control the crystallization of organic compounds [9–12] and the self-assembly of supramolecular systems in the design of new materials [13–15], and has received an increasing recognition in biomolecular engineering [16].

In the drug design area, halogenation of pharmacologically active compounds has traditionally been used as a common strategy to increase their metabolic stability and lipophilicity. Today, up to 20% of drugs on the market and up to a quarter of those in the development pipeline are halogenated, with even higher figures for the compounds in high-throughput drug screens (up to 50%) [17]. The large proportion of halogenated drugs in clinic and clinical trials implies the existence of halogen bonds in drug–target complexes. Although some early studies had shed light on the attractive interactions between ligands' halogens and proteins' oxygens [18, 19], the role and significance of halogen bonds in recognition and binding of ligands by biological receptors were only very recently "rediscovered" by pharmacologists and biologists in investigation of high-resolution crystal structures.

A full understanding of how halogenated molecules bind to biological substrates could open the door to new and more effective approaches to drug development, as well as to the rationalization of the adverse effects of some chemicals to which humans are commonly exposed [20]. Up to now, however, only limited works have been addressed on the functionality of halogen bonds acting in protein–ligand interactions. These works can be divided into three aspects in terms of the research methods and strategies: crystallographic study, structural survey, and theoretical analysis. Based upon this division, in this review we present a comprehensive discussion on the structural basis and energetic profile of halogen bonds in protein–ligand complexes; we start by briefly describing the principle and history of halogen bond. Then, this discussion will be returned to each research aspect of halogen bonds functioning to protein–ligand interactions. The last, we introduce a special halogen bond form named halogen-water-hydrogen bridge, which can serve as mediators to modulate the recognition and binding of halogenated ligands by their protein receptors.

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1389-5575/10 $55.00+.00

WHAT IS HALOGEN BOND?

The halogens in organic and inorganic compounds are known to polarize along their covalent bonds to generate an electropositive crown Fig. (**1a**). and thus act as a Lewis acid to pair with Lewis bases, such as oxygen, nitrogen, sulfur, and even aromatic ring. These electrostatic pairs, originally called charge-transfer bonds [4], are now referred as halogen bonds to underscore their shared similarities with hydrogen bonds in strength and directionality [7]. But here a question would be arisen, that is, why the generally electronegative halogens can generate an electropositive crown at their top to attractively interact with the electron donors? According to the "σ-hole" theory suggested by Murray and Politzer *et al.* [21–26], the valence electron orbits of covalent halogens, unlike that of oxygen and nitrogen, are almost in unhybridization state with the $s^2 p_x^2 p_y^2 p_z^1$ electronic configuration. As seen in Fig. (**1b**), the *s*-orbital, with slight *p*-hybridization component, possesses a pair of electrons and nearly symmetrically distributes around the halogen nuclear; two *p*-orbitals, p_x and p_y, are perpendicular to *z*-axis and each filled with two electrons; the remaining a half-filed *p*-orbital, p_z, possesses a electron that is σ-bonded with carbon. As a result, an electron hole occurs at the region of p_z opposite to the bonded carbon, giving rise to an electropositive crown at the outermost portion of the covalent halogen.

THE BRIEF HISTORY OF HALOGEN BOND

The first report on the ability of halogens to form well-defined adducts with electron donors dates back to 1863 when Guthrie described the formation of the $NH_3 \cdots I_2$ complex [2]. In 1896, Remsen and Norris proved the general tendency of amines interacting with bromine and chlorine [3]. More than 50 years later, Benesi and Hildebrand observed a typical case of molecular complexes formed from iodine and aromatic hydrocarbons [27]. These intermolecular interactions were sometimes called as "charge transfer" or "electron donor–acceptor" bonds, and Mülliken [28], and later Flurry [29], developed theoretical tools for describing them. Bent further pointed out that such interactions are ubiquitous and through which many organic halides can form noncovalent complexes with electron donor species [30]. The first use of the term "halogen bond", according to Politzer *et al.* [8], was found in the publication of Dumas *et al.* [31]. After then, the concept of halogen bond was progressively recognized by chemists, biologists, and material scientists, and also, a series of reviews relating to this topic were published to highlight the significance of halogen bond in molecular science [6–8, 20]. In recent years, halogen bonds have attracted a rapidly increasing interest in various areas, such as crystallography [9–12], supramolecular science [13–15] and biomolecular engineering [16], and numerous theoretical and experimental works have been addressed for ascertaining the geometric characteristics and energetic profile of halogenic interactions involved in different chemical and biological contexts.

CRYSTALLOGRAPHIC STUDY

Although a large number of crystal structures of protein–ligand complexes showing potential halogen bonds at the binding interfaces have been determined in past decades, only a very few of them were recently rediscovered and investigated. The most representative example is the protein kinase–inhibitor complexes of which the halogen bonds were systematically discussed in two reviews [32, 33]. In a perspective, by visual inspection of several high-resolution crystal structures Liao first claimed the importance of halogen bonds in improving binding affinity and specificity of inhibitors to cyclin-dependent kinase 2 (CDK2), the one of most well characterized kinases [32]. Voth and Ho lunched a comprehensive study to explore the role of halogen bonds in inhibitor recognition and binding by protein kinases [33]. In this study, twelve single crystal structures of protein kinase complexes with halogenated ligands were investigated in detail, and a favorable profile for a halogen bond acceptor as one which presents a concave or mostly concave surface to the halogen was defined to characterize the behavior of halogen bonds at the kinase–inhibitor interfaces. In addition, the short O···Cl and S···Cl contacts have also been re-found in crystal structures of the complexes between the human Xα factor and trichloro substituted inhibitor by Metrangolo *et al.* [7].

In several early studies, the I···O interactions were observed at the binding interfaces of transthyretin (TTR) with its selective ligands, including the thyroid hormone thyroxin (T4) [18, 19] and the pentabromophenol (PBP) [34]. These

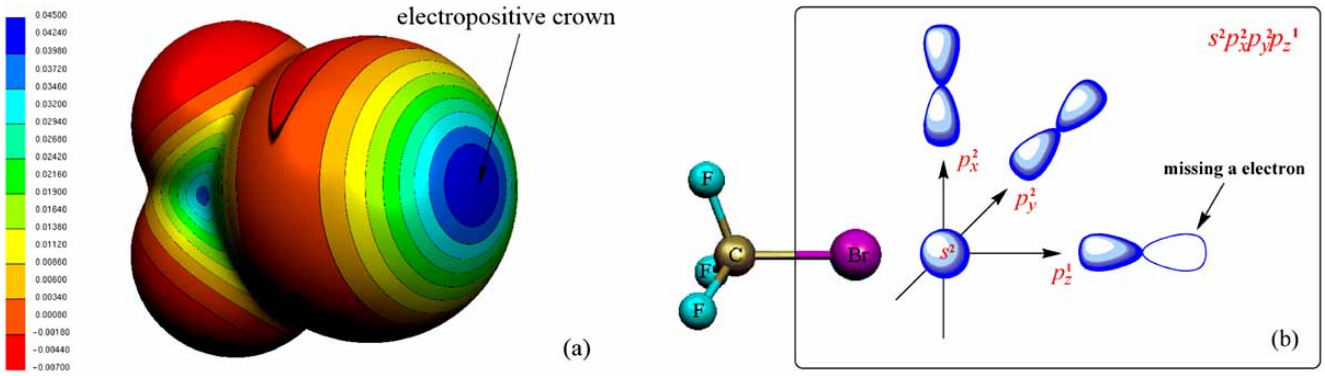

Fig. (1). (a) The molecular electrostatic potential, in Hartrees, at the 0.001 electrons $Bohr^{-3}$ isodensity surface of CF_3Br. This figure was modified from the Fig. **3**. in ref. [21] **(b)** Decomposition of the valence electron orbits of bromine in CF_3Br.

findings provided the first crystallographic evidence for the active role of halogen bonds in ligand recognition and binding. In recent years, the halogen bonds acting as mediator to modulate protein–ligand interactions have attracted increasing attention in crystallography community. To our knowledge, there are three experimental works that definitely mentioned the halogen bonds affecting ligand binding. Himmel and co-workers reported several high-resolution crystal structures for HIV-1 reverse transcriptase (RT) complexed with three pyridinone derivatives, and found that an iodine atom on the pyridinone ring of both inhibitors that interacts with the main-chain carbonyl oxygen of RT Tyr188 plays important roles in pyridinone derivatives inhibiting both wild-type and drug-resistant RTs Fig. (**2**) [35]. At nearly the same time, Battistutta *et al.* presented several crystal structures of the complexes between protein kinase CK2 and its selective inhibitors of tetrabromobenzimidazole derivatives and showed that the halogen bonds are established with the backbone of Glu114 and Val116 in the hinge region of CK2 [36]. Later, Jiang *et al.* synthesized and characterized two diastereomers of cephalomannine analogue, the depolymerization inhibitors of tubulin [37]. Glide analysis demonstrated that each model of the bound complexes for the two diastereomers presents a single well-defined halogen bond from one of the ligand's bromines to Glu22 and Asp26 near the N-terminus of α-tubulin, respectively.

Nevertheless, although halogen bonds have been highlighted to be a reasonable substitutor of hydrogen bonds in many chemical and biological systems [7, 16, 20, 38, 39], there was at least an experimental evidence showing that a hydrogen bond cannot functionally replaced using a halogen bond in the active site of ketosteroid isomerase [40], implying that the halogen bond is not an omnipotent tool to cover all the functional roles of other noncovalent forces.

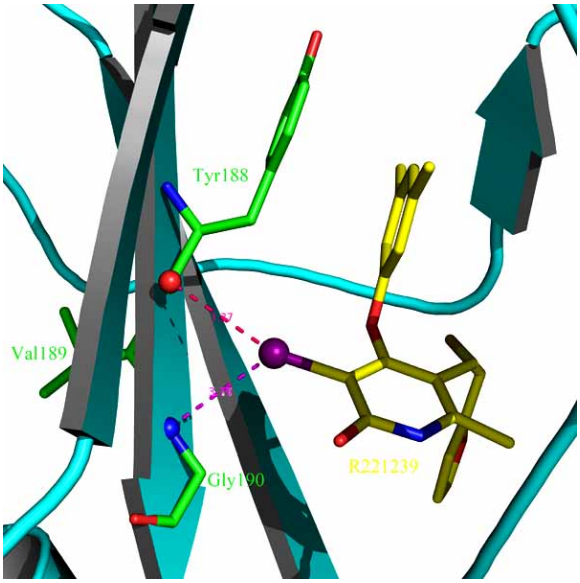

Fig. (2). Crystallographic evidence for halogen bond interactions of inhibitor R221239 with the HIV-1 RT (PDB: 2be2). The iodine on pyridinone ring of R221239 makes good main-chain contacts with residues Tyr188-Gly190, including a possible halogen bond and an electrostatic interaction with the Tyr188 carbonyl O and Gly190 amide N, respectively.

STRUCTURAL SURVEY

With the number of solved biomolecule 3D structures growing up rapidly in recent years, extraction and mining of biologically structural information based on statistical approach have become possible. Ho's group has cast the pioneering work towards the direction of statistical analysis of biological halogen bonds [41], in their study the geometric characteristics of all the halogen bonds extracted from the Protein Data Bank (PDB) [42] were surveyed comprehensively, revealing that halogen bonds are potentially stabilizing inter- and intra-molecular interactions that can affect ligand binding and biomolecular folding. Further, they proposed a novel concept as orthogonal molecular interactions between halogen and hydrogen bonds, that is, the halogen bonds are shown to be geometrically perpendicular to and energetically independent of hydrogen bonds that share a common carbonyl oxygen acceptor [43]. By structural survey and electrostatic potential analysis they demonstrated that this orthogonality is prevalent in protein–ligand complexes, suggesting that introduction of halogen bonds could be a strategy to improve ligand affinities without disrupting existing structurally important interactions. Interestingly, the orthogonality exists not only between the halogen and hydrogen bonds which share a common acceptor, but also in the cases where halogen atoms form a halogen bond in the "head on" orientation and a hydrogen bond in the "side on" fashion simultaneously [44]. Recently, Kortagere *et al.* have discussed the implications of halogenated ligands interacting with amino acids for structure–activity and structure–toxicity relationships, and revealed distinct patterns with respect to the nature and structural characteristics of halogen interactions with specific types of atoms and groups in proteins [45].

According to a recent survey [46], the PDB (December 2008 release) contains over 1000 structures in which the ligands are halogenated. Applying the criteria of (i) X···O distances (where X = Cl, Br, and I) less than the sums of their respective van der Walls radii and (ii) ∠(C–X···O) larger than 140° to analyze these structures 154 protein–ligand complexes were identified to show potential halogen bonds at their binding interfaces, leading to a total of 248 distinct X···O interactions (117 Cl···O, 54 Br···O, and 77 I···O for a total of 248 X···O). A brief summary of these halogen bonds is tabulated in Table **1**.

THEORITICAL ANALYSIS AND MODELING

Nowadays, two computational approaches are available for theoretical investigation of biological halogen bonds, *i.e.* molecular modeling and quantum mechanics. The molecular modeling employs empirical methods to construct the complex models of halogenated ligands with protein receptors, and to predict the potential halogen bonds in the complexes. For example, Gopalakrishnan *et al.* used docking and pharmacophore screening tools to examine the binding of deoxythymidine monophosphate (dTMP) analogues in the active site of thymidine monophosphate kinase ($TMPK_{mt}$) of *Mycobacterium tuberculosis* [47]. In this study, the crystal structure of $TMPK_{mt}$ bound with dTMP was adopted as receptor template, and 20 constructed dTMP analogues were in turn docked into the binding pocket of $TMPK_{mt}$ by using the

Table 1. A Brief Summary of the Halogen Bonds Observed at Protein–Ligand Interfaces

Halogen bond	Number	The average value and standard deviation of geometric parameters	
		bond length	bond angle
C–Cl···O	117	2.98±0.28	157.09±10.74
C–Br···O	54	3.17±0.16	160.05±11.94
C–I···O	77	3.28±0.15	161.75±10.19

GOLD program. The docking results showed that Br at the thymine C_5 of dTMP analogues forms a typical halogen bond with the backbone carbonyl oxygen of $TMPK_{mt}$ Phe36 ($Br^{\delta+}\cdots{}^{\delta-}O$=C, 3.01 Å); this interaction, together with several water-mediated cooperative networks and weak hydrogen bonds, contributes significantly to the stability of $TMPK_{mt}$–dTMP analogue complexes. Another report focusing on the molecular modeling of halogen bonds in protein–ligand complexes was recently published by Cheng *et al.* [48]. They employed virtual screening to investigate the binding modes of 135000 compounds with LXR*β* ligand-binding domain (LBD), and found about 247 compounds with docking poses positioning a halogen atom within 4.5 Å of LBD His435, indicating a potential halogen–nitrogen/oxygen interaction to stabilize agonistic conformation.

Although the molecular modeling technique can rapidly construct and analyze a large number of halogenated ligands binding to their cognate receptors, this approach is incapable of accurately predicting halogen bonds in complexes and providing the detailed information about halogen bond character at the electronic structure level. Alternatively, the quantum mechanics, serving as a complementary approach, has been broadly used to study the geometric and energetic characteristics of halogen bonds in small molecule, supramolecule, and biomolecule systems. Since protein–ligand complexes are quite large and comprise numerous atoms and groups, it is only possible to investigate a small model system (which mimics the local structure and environment around the studied halogen bonds) using quantum mechanical methods. Previously, we have addressed a series of works on this area and found that the strength of halogen bonds is influenced by various factors, including geometries, substituent groups, local environments, long-range interactions, etc. [49–53]. Recently, Riley *et al.* have successfully adopted halobenzene–formaldehyde complexes and bromobenzenes/bromopyrimidines–acetone complexes to ascertain the potential energy curves and electrostatic potential profiles of biological halogen bonds [54, 55].

Very recently, Lu *et al.* proposed a new strategy that uses two-layer ONIOM-based QM/MM scheme to investigate the halogen bonds across the binding interfaces of protein–ligand complexes [46]. Using this method 9 protein complexes with halogenated ligands were systematically analyzed and the halogen bonds in these systems were found to be approximately comparable in strength and directionality to classical hydrogen bonds, indicating that the halogen bond is promising as a novel interaction for rational drug design. To best of our knowledge, this work is the first use of quantum mechanics method to study halogen bonds in whole biomolecular systems.

EXTENSION OF CLASSICAL HALOGEN BOND

Our group has been dedicating to exploit special forms of halogen bonds in biomolecules. Recently, we have described a novel halogen-involved interaction that we named halogen-water-hydrogen (XWH) bridge to extend the application of classical halogen bond [56]. The XWH bridge can be regarded as one hydrogen bond in a water-mediated hydrogen bond bridge to be replaced by a halogen bond. Preliminary analysis revealed that the XWH bridges are more thermodynamically stable than other water-involved interactions, and this stability is further enhanced by the cooperation of the halogen bond with hydrogen bond. Structure survey demonstrated that this putative interaction indeed exists in biological environment and can serve as local stabilizer, chain binder, and ligand assistor to stabilize the advanced structures of proteins and nucleic acids, and to mediate their interactions with small ligands. For example, the kinase, c-Jun N-terminal kinase (JNK), in complex with its selective inhibitor was extracted from the PDB, showing a typical XWH bridge across their binding interface Fig. (**3**). Further, the geometric characteristics and energy landscapes of 9 structurally diverse protein–ligand complexes involving at least

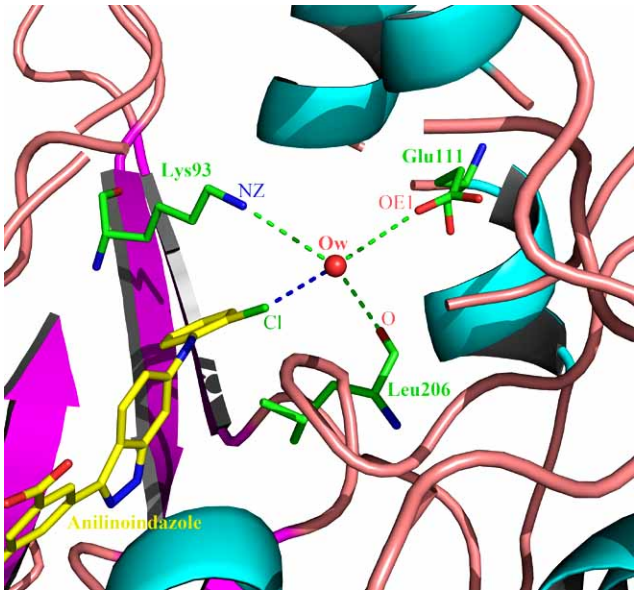

Fig. (3). A XWH bridge serving as ligand assistor presents at the binding interface of c-Jun N-terminal kinase (JNK) with its selective inhibitor of anilinoindazole (PDB: 2b1p).

one XWH bridge at their interfaces were analyzed in detail using two-layer ONIOM method and atoms in molecule (AIM) theory, the results obtained from the high-level quantum mechanical calculations definitely declared the existence and stability of XWH bridges at protein–ligand interfaces [57].

In addition, fluorine atoms in ligands, albeit they cannot form the traditional halogen bonds, were also observed by us to frequently contact with various kinds of atoms in proteins, leading to weak closed-shell interactions to confer the specificity and affinity for protein–ligand binding [58].

SUMMARY

Although short halogen–oxygen/nitrogen/sulfur contacts have been observed in protein–ligand complexes more than three decades [18, 19], they were only very recently rediscovered as halogen bonds to stress their shared similarities with hydrogen bonds. Crystal structure surveys uncovered a considerable number of halogen–oxygen interactions located at the binding interfaces of proteins with their cognate ligands [41], these interactions were defined as halogen bonds and were subsequently found to show a significant orthogonality with the hydrogen bonds that share a common carbonyl oxygen acceptor [43]. Crystallographic evidences demonstrated that halogen bonds play active roles in the recognition and binding of HIV-1 RT, CK2, and protein kinases by their selective inhibitors [33–36]. Quantum mechanics and molecular modeling clearly revealed the geometric preference and energetic profile of halogen bonds in different chemical and biological contexts [46–48]. These works put the first step towards the direction of the functionality of halogen bonds in protein–ligand interactions, and shed light on the application of halogen bond as a novel tool to rational drug design.

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Received: December 16, 2009 Revised: March 19, 2010 Accepted: March 20, 2010