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Insights into halogen bond-driven enantioseparations

Paola Peluso[a,*], Victor Mamane[b,*], Emmanuel Aubert[c], Alessandro Dessì[a], Roberto Dallocchio[a], Antonio Dore[d], Patrick Pale[b], Sergio Cossu[e]

[a] Istituto di Chimica Biomolecolare ICB, CNR, UOS di Sassari, Traversa La Crucca 3, Regione Baldinca, I-07100 Li Punti, Sassari, Italy
[b] Institut de Chimie de Strasbourg, UMR 7177, Equipe LASYROC, 1 rue Blaise Pascal, BP 296 R8, 67008 Strasbourg Cedex, France
[c] Cristallographie, Résonance Magnétique et Modélisations (CRM2), UMR CNRS 7036, Université de Lorraine, BP 70239, Bd des Aiguillettes, 54506 Vandoeuvre-les-Nancy, France
[d] ISPA, CNR, UOS di Sassari, Traversa La Crucca 3, Regione Baldinca, I-07100 Li Punti, Sassari, Italy
[e] Dipartimento di Scienze Molecolari e Nanosistemi, Università Ca' Foscari di Venezia, Via Torino 155, I-30172 Mestre Venezia, Italy

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ABSTRACT

Although the halogen bond (XB) has been so far mainly studied *in silico* and in the solid state, its potential impact in solution is yet to be fully understood. In this study, we describe the first systematic investigation on the halogen bond in solvated environment by high-performance liquid chromatography (HPLC). Thirty three atropisomeric polyhalogenated-4,4′-bipyridines (HBipys), containing Cl, Br and I as substituents, were selected and used as potential XB donors (XBDs) on two cellulose-based chiral stationary phases (CSPs) containing potential XB acceptors (XBAs). The impact of the halogens on the enantiodiscrimination mechanism was investigated and iodine showed a pivotal role on the enantioseparation in non-polar medium. Electrostatic potentials (EPs) were computed to understand the electrostatic component of CSP-analyte interaction. Moreover, van't Hoff studies for ten HBipys were performed and the thermodynamic parameters governing the halogen-dependent enantioseparations are discussed. Finally, a molecular dynamic (MD) simulation is proposed to model halogen bond in polysaccharide-analyte complexes by inclusion of a charged extra point to represent the positive 'σ-hole' on the halogen atom. On the basis of both experimental results and theoretical data, we have profiled the halogen bond as a chemo-, regio-, site- and stereoselective interaction which can work in HPLC environment besides other known interactions based on the complementarity between selector and selectand.

1. Introduction

The term *halogen bond* (XB) describes the intermolecular interaction occurring between a Lewis base and a halogen atom which behaves like Lewis acid, according to the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) (*a halogen bond occurs when there is evidence of a net attractive interaction between an electrophilic region associated with a halogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity*) [1]. The electrophilic nature of halogens is explained by the anisotropic distribution of the electron density around the halogen atoms (Fig. 1) [2].

Long since studied [3,4] due to its unique characteristics of selectivity and interaction geometry, currently, the XB is considered a relevant evidence in many recognition processes [5,6], with a strong impact in crystal engineering [7,8] and medicinal chemistry [9], and a growing interest in fields related to magnetics, optics and electronics [5]. Moreover, several *in silico* studies have been dedicated to the topic [10,11]. In the last few decades, the investigations on XB have mainly been focused on its description in vacuum and in solid state. On the contrary, despite the importance and the capability in biochemical recognition [12] and in synthetic chemistry [13], the behaviour of XB in solution has received less attention [14–16]. To date, halogen bond in solution has been studied by using techniques as UV [17], IR [18], EPR [19] and significantly NMR [20]. The potentiality of the XB has been investigated in analytical chemistry as well. Indeed, Jin and co-workers have shown that a C—I···Cl^- interaction is active in the solid phase extraction of perfluorinated iodoalkanes (PFIs) from *n*-hexane by using a strong anion exchange (SAX) sorbent [21,22].

Recently, the enantioseparation of polyhalogenated 4,4′-bipyridine (HBipy) **1**, performed by our groups (Fig. 2A), proved that the XB can be active also in high-performance liquid

* Corresponding authors.
E-mail addresses: p.peluso@icb.cnr.it (P. Peluso), vmamane@unistra.fr (V. Mamane).

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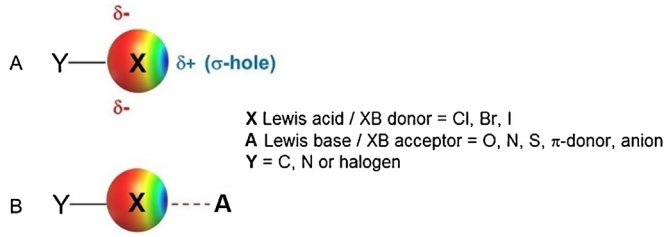

Fig. 1. General description of halogen bond.

chromatography (HPLC) environment [23]. Specifically, cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) showed to be suitable as chiral selector for recognizing the enantiomers of the iodinated HBipy **1**. This result appeared to be related to the ability of iodine as XB donor (XBD) to form linear interactions with carbonyl oxygen atoms (C=O) and aromatic π systems as XB acceptors (XBAs) (Fig. 2B). The carbonyl-halogen bonds have been widely studied in medicinal chemistry, which showed to have a relevant role in protein-pharmacophore complexes [24,25]. In nature, halogenated XB donors are not unusual and the selective binding of the hormone thyroxine (T4) to its transporter protein transthyretin involves iodine-carbonyl oxygen interaction, for instance [26].

On this basis, in the present study we describe the first systematic investigation on the halogen bond in solvated environment performed by HPLC using two cellulose-based CSPs which contain potential XBAs and chiral guests bearing halogen substituents able to behave as XBDs. With the aim to know how the XB interaction works in LC chromatography, at both chiral and achiral levels, HBipys **1**-**33** (Fig. 2A, Table 1) were designed, selected and synthesized through focused procedures [27–31]. Thus, firstly, the influence of the structure of CSP, halogen substitution pattern of the analytes and mobile phase (MP) polarity on the effectiveness of halogen-dependent enantioseparations were evaluated by means of the changes of the chromatographic responses upon structural variations. Secondly, electrostatic potentials (EPs) were computed to understand the electrostatic component of CSP-analyte interaction [32]. Thirdly, a study at variable temperature was performed to determine the thermodynamic parameters associated with the enantioseparation process. Moreover, a molecular dynamic (MD) simulation is proposed to model halogen bond in polysaccharide-analyte complexes by inclusion of a charged extra point to represent the positive 'σ-hole' on the halogen atom [33,34]. Finally, a gas chromatographic screening (GC) was performed by using two chiral capillary columns with the aim to verify if halogen-dependent enantioseparations can occur in a different chromatographic medium.

2. Experimental

2.1. Chemicals

The syntheses of compounds **3**, **25**, and **30**-**33** together with the ^{1}H and ^{13}C NMR, and HRMS spectra, and the names of HBipys **1**-**33** are available in the Supplementary data. Compounds **1**, **2**, **4**-**24**, and **26**-**29** were synthesized as reported [27,28,31].

2.2. Chromatography

An Agilent Technologies (Waldbronn, Germany) 1100 Series HPLC system (high-pressure binary gradient system equipped with a diode-array detector operating at multiple wavelengths (220, 254, 280, 360 nm), a programmable autosampler with a 20 μl loop, and a thermostatted column compartment) was employed for both analytical and multimilligram

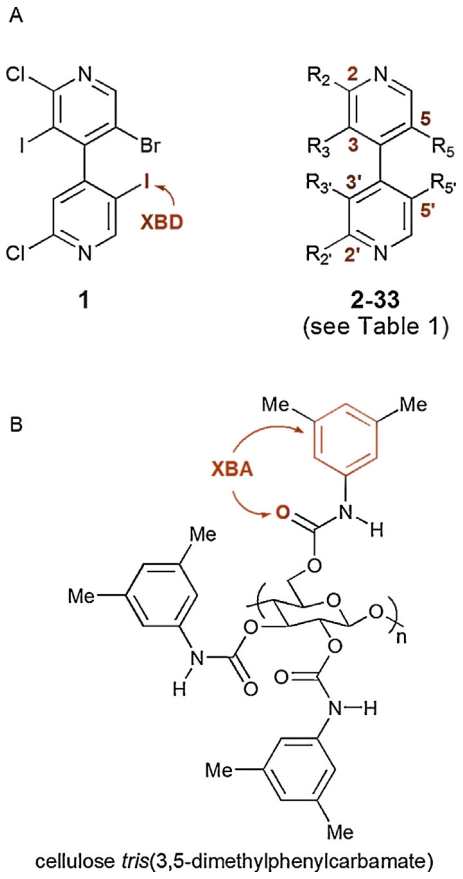

Fig. 2. A. Structures of the polyhalogenated 4,4′-bipyridines (HBipys) **1**-**33**; B. Cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) (Lux-Cellulose 1).

separations. Data acquisition and analyses were carried out with Agilent Technologies ChemStation Version B.04.03 chromatographic data software. The UV absorbance is reported as milliabsorbance units (mAU). Lux Cellulose-1 (Phenomenex, USA), Chiralcel OD-H (Daicel, Tokyo, Japan) (cellulose tris-3,5-dimethylphenylcarbamate; 5 μm), Lux Cellulose-2 (cellulose tris-3-chloro-4-methylphenylcarbamate; 5 μm, Phenomenex, USA), Chiralpak IC (cellulose tris-3,5-dichlorophenylcarbamate; 5 μm) and Chiralpak IA (amylose tris-3,5-dimethylphenylcarbamate; 5 μm) (Chiral Technologies Europe, Illkirch, France) were used as chiral columns (250 × 4.6 mm). HPLC grade ethanol (EtOH), *n*-hexane (Hex), *n*-heptane, methanol (MeOH), 2-propanol (IPA), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (Taufkirchen, Germany). The retention factor (k) was determined as $k = (t_R - t_0)/t_0$, where t_R is the retention time for the eluted enantiomer; k_1 is the retention factor of the first-eluted enantiomer. The separation factor (α) was calculated as $\alpha = k_2/k_1$. The resolution (R_s) was determined as $2(t_{R2} - t_{R1})/(W_1 + W_2)$ where W is the basewidth of peak. Dead time (t_0) was measured by injection of tris-*tert*-butylbenzene (Sigma-Aldrich, Taufkirchen, Germany) as a non-retained compound [35]. Analyses were performed in isocratic mode at 22 °C. The flow rate (*FR*) was set at 0.8 ml/min for analytical separations. The enantiomer elution order (EEO) was determined for compounds **1**-**6**, **8**, **10**-**12**, **14**-**16**, **19**-**24**, **29**, **30**, **32**, and **33** by injecting enantiomers of known absolute configuration. The absolute configuration was assigned on the basis of X-ray diffraction (XRD) (Supplementary data), and as reported [27,28,31]. The van't Hoff experiments were conducted at 10, 15, 20, 25, 30 and 35 °C in a thermostatted column chamber equipped with a cooling system. When the temperature was changed, the column was allowed to equilibrate for 1 h before injecting the samples. Thermodynamic