Article

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Abstract

Halogen bonds have recently been introduced as ideal to transport anions across lipid bilayer membranes. However, activities obtained with small transporters were not impressive, and cyclic arrays of strong halogen-bond donors above a calix[4]arene scaffold gave even weaker activities. Here, we report that their linear alignment for anion hopping along transmembrane rigid-rod scaffolds gives excellent activities with an unprecedented cooperativity coefficient m = 3.37.

Reference

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Transmembrane Halogen-Bonding Cascades

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ABSTRACT. Halogen bonds have recently been introduced as ideal to transport anions across lipid bilayer membranes. However, activities obtained with small transporters were not impressive, and cyclic arrays of strong halogen-bond donors above a calix[4]arene scaffold gave even weaker activities. Here, we report that their linear alignment for anion hopping along transmembrane rigid-rod scaffolds gives excellent activities with an unprecedented cooperativity coefficient $m = 3.37$.

Halogen bonds originate mainly from the localized positive charge density, the so-called σ-hole, that appears best on highly electron-deficient halogen atoms, particularly iodines.[1] However, other contributions to halogen bonds should also not be underestimated (polarization, charge transfer, etc).[1] Halogen-bond donors can interact with electron-rich sites such as lone pairs on oxygens or nitrogens, or with anions. Like hydrogen bonds, halogen bonds are strong, particularly in non-polar environments, and excel with unique directionality. Halogen bonds have attracted much interest in theory and are quite routinely used in crystal engineering.[1] More recently, several examples for anion binding[2] and catalysis[3] with halogen bonds have appeared, and applications in chemical biology become more frequent.[4]

Anion transport[5] with halogen bonds has been reported last year to be particularly attractive because, unlike hydrogen-bond donors, halogen-bond donors are intrinsically hydrophobic.[6] For this reason, anion transport is already possible with very small molecules such as pentafluoroiodobenzene **1** or even trifluoroiodomethane, a gas with a boiling point of -22 ^{0}C (Figure 1, Table 1). For comparison, anion-π interactions with hexafluorobenzene **2** were insufficient for anion transport in this minimalist format. However, the positioning of four tetrafluoroiodobenzyls in a cyclic array above the calix[4]arene scaf-

fold in **3** gave much weaker activities.[7] This suggested that, contrary to the weaker anion-π interactions in **4**, anion binding by halogen bonds in **3** is thermodynamically as well as kinetically too strong for transport.[7] To promote anion transport rather than binding, we concluded, cyclic arrays of halogen-bond donors would have to be unrolled into linear arrays for transmembrane anion hopping, and decided to synthesize and evaluate the series **5-12**.
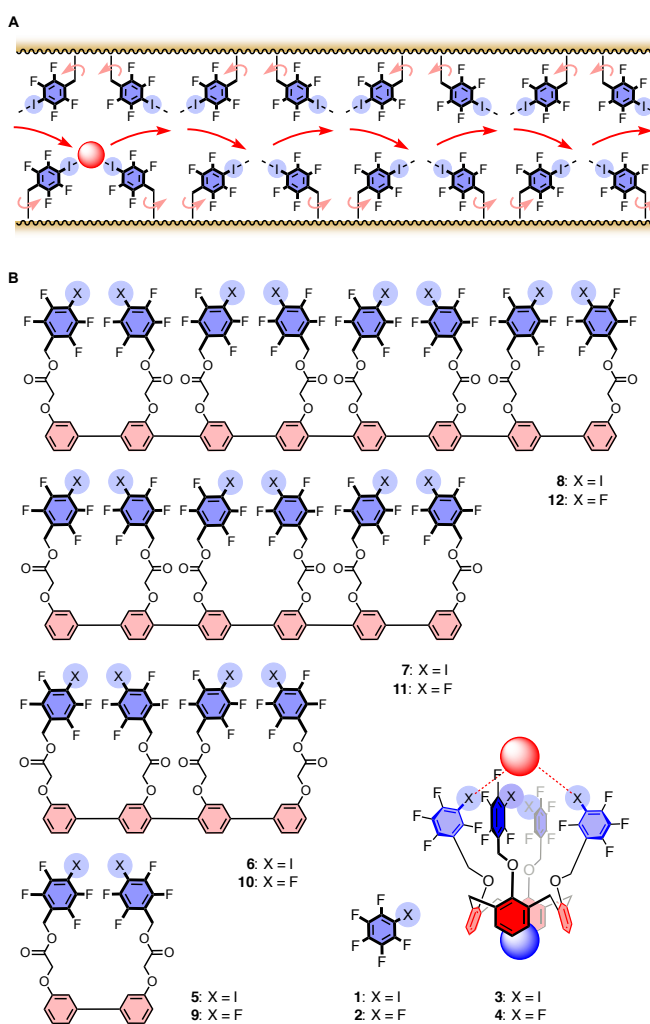
Figure 1. (A) Schematic representation of anion hopping along transmembrane halogen-bonding cascades. (B) Halogen bond donors as monomers (**1**),[6] in cyclic arrays (**3**),[7] and in linear arrays (**5-8**), together with the controls for complementary anion-π interactions (**2, 4, 9-12**).

Table 1 Ion transport activity of halogen-bond donors **1-12**.[a]

Cpd [b]	EC_{50} (μM) [c]	EC_{50}^{N} (μM) [d]	n [e]
1 [f]	260	260	3.6
2 [f]	>2000	-	-
3 [g]	~1000	4000	0.7
4 [g]	25	-	1.1
5	9.2 ± 0.8	18.4	1.0 ± 0.2
6	0.72 ± 0.04	2.9	1.0 ± 0.1
7	0.13 ± 0.01	0.78	1.3 ± 0.1
8	0.11 ± 0.02	0.88	0.8 ± 0.1
9	32.5 ± 3.4	65.0	1.1 ± 0.1
10	12.6 ± 3.0	50.4	0.8 ± 0.1
11	1.3 ± 0.5	7.8	0.5 ± 0.1
12	2.9 ± 0.4	23.2	0.6 ± 0.1

[a] Determined from HPTS assay in EYPC-LUVs⊃HPTS (100 mM NaCl, 10 mM HEPES, pH 7, external NaOH pulse).[11] [b] Compounds, see Fig. 1. [c] Effective concentration needed to see 50% activity. [d] Effective concentration per monomer unit in an oligomer. [e] Hill coefficient. Concentration. [f] From ref. 6. [g] From ref. 7.

Early work on the complementary hydrogen-bonded chains[8] has identified *p*-oligophenyls as privileged scaffolds.[9,10] The halogen-bonding cascades in homologs **5-8** were obtained by coupling of the original *p*-oligophenyls with carboxylic acids along the scaffold with tetrafluoroiodobenzyl chloride, bromide or iodide.[11] The control series **9-12** to compare for anion-π interactions was obtained analogously.

Ion transport activity was determined with the HPTS assay under routine conditions.[6,7,11] In this assay, large unilamellar vesicles (LUVs) composed of egg yolk phosphatidylcholine (EYPC) are loaded with 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS). Then a pH gradient is applied with a base pulse, the transporter is added, and the ability of the transporter to accelerate the dissipation of the pH gradient is measured with the intravesicular, ratiometric pH probe. The conditions were selected to report, presumably, on Cl⁻/OH⁻ antiport.

The new linear arrays **5-12** were analyzed under conditions identical to the ones used for monomers and cyclic arrays **1-4**.[6,7] For all compounds, dose response curves were recorded. Their Hill analysis gave the effective concentrations EC50, that is the monomer concentration needed to reach 50% of the activity at saturation. Routine control experiments confirmed the absence of nonspecific dye leakage during anion transport and afforded a length-independent, moderate selectivity for nitrate and chloride on a very weak anti-Hofmeister anion selectivity background.[11]

Transporters **5-8** with linear halogen-bonding arrays were 3.5-26 times more active than their homologous controls **9-12** for anion-π interactions (Table 1). Gratifyingly, the transport activities increased with increasing length of the rigid-rod scaffold. The EC50^N, that is the EC50 per monomer N, decreased for both "halogen-bond" transporters **5-8** and "anion-π" transporters **9-12**. Saturation was reached around scaffolds long enough to enable transmembrane[10] linear arrays (Table 1).[12] According to equation (1),[13]

$$EC50 \propto N^{-m} \qquad\qquad (1)$$

the dependence of the EC$_{50}$ on the number N of monomers per oligomer gave a cooperativity coefficient $m = 2.13$ for anion-π transporters **9-12**. This value is already slightly above the usual range for intrinsic multivalency contributions with oligomers and polymers ($1 < m < 2$).[13] With linear arrays of halogen-bond donors in **5-8**, $m = 3.37$ was found. This exceptionally high value provided quantitative evidence for the existence of transmembrane halogen-bonding cascades for cooperative anion transport as outlined in Figure 1A. In the best transporter, i.e., octamer **8**, halogen-bonding cascades gave 2360 times higher activity compared to monomer **1**. Presumably, this anion hopping occurs within bundles of transmembrane rigid-rod scaffolds, either intra- or intermolecularly.[14] Hill coefficients $n \sim 1$ are not in contradiction with this assumption (Table 1). They only demonstrate that these bundles are thermodynamically stable.[15]

In summary, this study on halogen-bonding cascades merges current topics with a comprehensive research program that has been launched in 1997.[8] The results fully confirm the power of transmembrane

rigid-rod scaffolds on the one hand and halogen bonds in the other hand to achieve efficient anion hopping across lipid bilayer membranes.

ASSOCIATED CONTENT

Supporting Information. Details on experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) (a) Metrangolo, P.; Meyer, F.; Pilati, T.; Resnati, G.; Terraneo, G. *Angew. Chem. Int. Ed.* **2008**, *47*, 6114-6127. (b) Laurence, C.; Graton, J.; Berthelot, M.; El Ghomari, M. J. *Chem. Eur. J.* **2011**, *17*, 10431-10444. (c) Huber, S. M.; Elisa Jimenez-Izal, E.; Ugalde, J. M.; Infante, I. *Chem. Commun.* **2012**, *48*, 7708-7710.

(2) (a) Mele, A.; Metrangolo, P.; Neukirch, H.; Pilati, T.; Resnati, G. *J. Am. Chem. Soc.* **2005**, *127*, 14972-14973. (b) Chudzinski, M. G.; McClary, C. A. Taylor, M. S. *J. Am. Chem. Soc.* **2011**, *133*, 10559-10567. (c) Caballero, A.; Zapata, F.; White, N. G.; Costa, P. J.; Félix, V.; Beer, P. D. *Angew. Chem. Int. Ed.* **2012**, *51*, 1876-1880.

(3) (a) Bruckmann, A.; Pena, M. A.; Bolm, C. *Synlett* **2008**, *6*, 900-902. (b) Walter, S.; Kniep, F.; Herdtweck, E.; Huber, S. *Angew. Chem. Int. Ed.* **2011**, *50*, 7187-7191.

(4) (a) Wilcken, R.; Zimmermann, M. O.; Joerger, A. C.; Boeckler, F. M. *J. Med. Chem.* **2013**, *56*, 1363-1388. (b) Hardegger, L. A.; Kuhn, B.; Spinnler, B.; Anselm, L.; Ecabert, R.; Stihle, M.; Gsell, B.; Thoma, R.; Diez, J.; Benz, J.; Plancher, J. M.; Hartmann, G.; Banner, D. W.; Haap, W.; Diederich, F. *Angew. Chem. Int. Ed.* **2011**, *50*, 314-318.

(5) (a) Chui, J. K.; Fyles, T. M. *Chem. Soc. Rev.* **2012**, *41*, 148-175. (b) Davis, J. T.; Okunola, O.; Quesada, R. *Chem. Soc. Rev.* **2010**, *39*, 3843-3862. (c) Davis, A. P.; Sheppard, D. N.; Smith, B. D. *Chem. Soc. Rev.* **2007**, *36*, 348-357. (d) Gale, P. A. *Acc. Chem. Res.* **2011**, *44*, 216-226.

(6) Vargas Jentzsch, A.; Emery, D.; Mareda, J.; Nayak, S. K.; Metrangolo, P.; Resnati, G.; Sakai, N.; Matile, S. *Nature Commun.* **2012**, *3*, 905.

(7) Vargas Jentzsch, A.; Emery, D.; Mareda, J.; Metrangolo, P.; Resnati, G.; Matile, S. *Angew. Chem. Int. Ed.* **2011**, *50*, 11675-11678.

(8) Weiss, L. A.; Sakai, N.; Ghebremariam, B.; Ni, C.; Matile, S. *J. Am. Chem. Soc.* **1997**, *119*, 12142-12149.

(9) Sakai, N.; Mareda, J.; Matile, S. *Acc. Chem. Res.* **2008**, *41*, 1354-1365.

(10) Transmembrane orientation of hydrophobically matching *p*-oligophenyl scaffolds has been confirmed by fluorescence depth quenching for many examples.[8,9]

(11) See SI.

(12) Saturation with hexamers rather than octamers[8,9] was reasonable considering that the rather long sidechains can reach out toward the surface of the membrane. Increasing vesicle concentrations also increased the EC50 (e.g., 1.25 mM EYPC: **7**, 700 nM; **8**, 1.75 µM).[11]

(13) Hennig, A.; Gabriel, G. J.; Tew, G. N.; Matile, S. *J. Am. Chem. Soc.* **2008**, *130*, 10338-10344.

(14) Direct evidence from functional studies supports that, not surprisingly, perfluoroiodoarenes self-assemble in lipid bilayers.[6]

(15) Bhosale, S.; Matile, S. *Chirality* **2006**, *18*, 849-856.