Anion-selective electrode based on a CH-hydrogen bonding bis-macrocyclic ionophore with a clamshell architecture

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ABSTRACT: CH-hydrogen bonding provides access to new building blocks for making macrocyclic ionophores with high degrees of preorganization and selective anion recognition. In this study, an anion-binding ionophore in the shape of a clamshell (ClS) was employed that is composed of two cyanostar (CNstar) macrocycles with preorganized cavities linked with a 12-carbon chain. This ionophore allows for anion complexation by CH-hydrogen bonding. The potentiometric performance of membrane-based ion-selective electrodes incorporating this ionophore was evaluated. Different membrane compositions were prepared to determine the optimum concentrations of the ionophore and lipophilic additive in the membrane. The optimized electrode had a slope of –58.2 mV per decade and demonstrated an anti-Hofmeister selectivity pattern toward iodide with a nanomolar detection limit. Electrospray ionization mass spectrometry was employed to study the relative association strengths of ClS with various anions. The observed mass peaks of the ion-ionophore complexes were found to be consistent with the potentiometric selectivity pattern of the corresponding electrodes. Overall, the selectivity of the electrode could be altered by using an ionophore in which the two CNstar macrocycles are linked together with a flexible 12-carbon chain to control the molecularity of the binding event.

Introduction

Designing ionophores for larger sized anions is challenging as a result of the complexity of synthesizing larger hosts with functionalities preorganized for binding weakly coordinating anions. The study of anion recognition with preorganized ionophores that rely on reversible binding interactions is a topic of great interest in supramolecular chemistry.[1] In the past, the design of anion-selective ionophores based on hydrogen bonding has mainly employed NH functionalities. However, computational studies have shown that CH groups on benzene have about half the strength of NH in forming hydrogen bonds with anions.[2,3] This discovery helped direct interest toward designing anion-selective ionophores based on pure CH hydrogen bonding.[4-10] Several neutral macrocycles that can complex anions based on CH hydrogen bonding have been studied by our groups for use in ion-selective electrodes (ISEs).[6, 11-13] While the electronegativity of the carbon is low compared to conventional hydrogen bond donors, it can be enhanced by introducing electron withdrawing groups.[14] Using an ionophore that is capable of forming CH hydrogen bonds with anions is more desirable than conventional ionophores that form hydrogen bonds based on NH donor sites. The NH groups in some of these ionophores are susceptible to protonation to generate a charged species, thereby, potentially diminishing the selectivity of the corresponding ISEs. Despite these advantages, deployment of anion-selective ionophores based on pure CH hydrogen bonding as the active component in ISEs is rare.[11-13]

Recently, Zahran et al.[13] introduced a highly selective perchlorate sensor based on the cyanostar (CNstar) ionophore (Figure 1a). CNstar is a toroid-shaped macrocycle with 10 convergent CH hydrogen bonding sites that provides size-selective interactions to anions by creating a 2:1 sandwich complex [15] (Figure 1b) reinforced by cofacial π–π stacking.[16] This structural organization leads to creation of a cavity in the host molecule capable of accommodating large anions traditionally considered to be weakly coordinating, such as perchlorate (ClO_4^-). The strength of the CH-based hydrogen bonds in CNstar are enhanced by the electron withdrawing nitrile groups, which polarize the CH donors to create a stronger partial positive charge on the hydrogen pointing into the cavity. The cavity diameter of CNstar is 4.5 Å, which matches nicely with the perchlorate diameter of 4.5 Å. The CNstar-based ISE demonstrated high selectivity towards perchlorate with a detection limit of 50 nM

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and a slope of −57.0 mV/decade over a linear range of 100 nM to 0.1 M.[13]

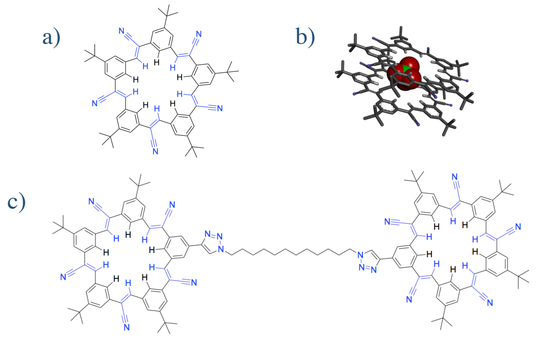

Figure 1. (a) Structure of cyanostar (CNstar); (b) Schematic of CNstar in a 2:1 complex with perchlorate (c) Structure of clamshell (ClS).

In the case of CNstar, the ability of the ionophore to form a sandwich-type complex with the anion is a crucial part of its performance. In modified CNstar-based macrocycles bearing bulky groups that prevent sandwich formation, binding energies with anions are halved.[17] Introduction of a linker of appropriate length and flexibility as a bridge between two host macrocycles is a well-established approach to manipulate the selectivity of receptors, such as bis-crown ether and double decker porphyrin ionophores.[18-21] There is also an entropic advantage provided by an ionophore that has two linked macrocycles compared to an ionophore containing a single macrocycle.[22-24]

To explore the effect of CNstar dimerization on formation of the sandwich-type complexes around anions, two CNstar molecules were connected using a 12-carbon chain bridge to develop the new ionophore, clamshell (ClS, Figure 1c).[25] This compound was previously used to investigate the mechanism of redox-driven molecular switching.[26] In the ClS design, the flexible 12-carbon chain can fold to allow the two CNstar units to complex anions in an intramolecular π-stacked sandwich. An alternative higher-order structure would involve two of the clamshell macrocycles coming together to form a 2:2 complex composed of two intermolecular sandwiches (*vide infra*). The clamshell has the same macrocyclic cavity as CNstar, but one of the phenylene rings on each macrocycle is functionalized with a 1,2,3-triazole connected to a bridging 12-carbon chain (Figure 1c). The presence of the triazole groups at each end of the carbon chain may alter the strength of the hydrogen bonding of the ionophore, although this would be a small change relative to the other nine unchanged CH donors.

Electrospray ionization mass spectrometry (ESI-MS) has proved useful in helping to understand the possible complexes formed within the polymeric membrane of ISEs.[27] ESI-MS spectra reflect the gas-phase, solvent-free structures and stoichiometries of molecules and macromolecules. On account of the gentle nature of the electrospray ionization process, the presence of noncovalent complexes in the gas-phase is readily detectable.[28-30] ESI-MS has been used to study the binding specificities, affinity, and stoichiometry of complexes at concentrations as low as picomolar.[31] This technique has also been used successfully in host-guest chemistry [32] in which both electronic effects and size-selectivity influence the formation, reactions, and stabilities of gas-phase complexes.[33-36].Recently, a meaningful correlation between the potentiometric selectivity of neutral carrier ionophores and ESI-MS relative peak intensity has been demonstrated.[29, 31] Thus, we are expecting a similar correlation to be useful when studying the CNstar-based clamshell.

Herein, we describe the performance of the clamshell ionophore for anion-selective electrodes. By tethering the macrocycles together in the ionophore, we hypothesized that the selectivity of the CNstar macrocycle toward size-matched anions like perchlorate would change in ClS. The response of different membrane compositions containing various ratios of ClS and the ion-exchanger, tridodecylmethylammonium chloride (TDMAC), was evaluated to optimize the selectivity of the ISE. In contrast to the parent CNstar-based ISE [13], we found that the selectivity when using ClS was shifted from perchlorate to iodide in the clamshell structure. The selectivity pattern of the clamshell ionophore toward several anions was also studied with ESI-MS to elucidate the correlation between the potentiometric selectivity and the coordination preferences of the ionophore. The ability of clamshell to form complexes was compared to that of the parent CNstar by both potentiometric and ESI-MS techniques. Overall, we find that the selectivity could be shifted from perchlorate to iodide in the clamshell structure paving the way for manipulation of the ionophore's molecularity to tune the selectivity of ion-selective electrodes.

Experimental Section

Reagents

The clamshell ionophore (ClS) was synthesized as described in reference.[25] All chemicals used for the preparation of ion-selective membranes, including 2-nitrophenyl octyl ether (NPOE), tridodecylmethylammonium chloride (TDMAC), and poly (vinyl chloride) (PVC), were purchased in Selectophore grade from Fluka (Ronkonkoma, NY). High purity sodium perchlorate, sodium chloride, sodium bromide, sodium nitrate, sodium salicylate, sodium iodide, sodium thiocyanate, and sodium hydroxide along with the buffer N-(2-hydroxyethyl) piperazine-N-(2-ethanesulfonic acid) (HEPES) and tetrahydrofuran (THF) were obtained from Sigma-Aldrich (St. Louis, MO). Deionized water of 18 MΩ•cm (E-Pure water purification system; Thermo Scientific, Marietta, OH) was used for all solution preparations and measurements.

Membranes and Electrode Preparation

The PVC-based ion-selective electrodes were prepared according to established procedures. Briefly, specific amounts of clamshell, TDMAC, PVC, and NPOE plasticizer (see Table 1 for composition; ratio of PVC to NPOE in the membrane was approximately 1:2) were dissolved in 1.5 mL of THF to prepare membrane cocktails. The cocktail was left at rest to release any air bubbles and then poured into a 22 mm-i.d. glass ring that was secured on a glass slide. The cocktail was left overnight at room temperature to allow THF to evaporate slowly. Finally, the formed 150–200 µm thick membrane was cut into small disks of 7 mm diameter using a cork borer to install onto Philips IS-561 (Glasbläserei Möller, Zurich, Switzerland) electrode bodies. A solution of 1×10^{-3} M KCl was used as the internal filling solution. Membranes were conditioned in 1×10^{-2} M solution of the anion for at least four hours before evaluating the potentiometric performance.

Potentiometric Measurements

All potentiometric measurements were performed at 22 ± 0.5 °C using the following cell assembly: Ag/AgCl|3.0 M KCl||1.0 M CH_3COOLi||sample solution| ISE membrane|1×10^{-3} M NaCl|Ag/AgCl. The cell potential was measured using an 8-channel SC-2345 interface (SCC-LP01, National

Table 1. Membrane composition and iodide response characteristics of different ISEs.

Membrane*	ClS wt%	TDMAC mol% ratio to ionophore	Slope I^- (mV/decade)
I	0	1 wt%	-63.7
II	1	25	-26.6
III	1	60	-49.8
IV	1	75	-58.2
V	1	100	-58.1
VI	1	160	-62.5
VII	2	25	-19.1
VIII	2	50	-49.2
IX	2	60	-59.1
X	2	75	-58.5
XI	3	60	-57.9

* All membranes are composed of ~33 wt% high molecular weight PVC, ~66 wt% of 2-nitrophenyl octyl ether (NPOE) as plasticizer, and the amounts of ClS and TDMAC listed in the table to make up the 100%.

Instruments, Austin, TX). This interface was connected to a PC computer using a PCI-6036E multifunction I/O data acquisition device. A custom-designed data acquisition script written in LabVIEW 7.0 (National Instruments) software was used to record the EMF measurements. A Fisher Scientific Accumet 915 pH/Ion meter, which was equipped with an Orion pH glass electrode (Beverly, MA), was used throughout for pH studies and buffer preparations. The potentiometric response of the clamshell-based electrodes was evaluated in 0.5 M HEPES buffer adjusted to pH 7.4 with NaOH. The lifetime of the clamshell-based electrodes was also studied over a period of sixty days. For the first sixteen days, the electrode was evaluated daily, and after that it was used on day 37 and day 60. In between measurements, the electrode was kept in 1 x 10^{-3} M NaCl solution. Before each measurement, the electrode was conditioned in 1 x 10^{-3} M NaI solution for at least two hours. The results were compared with the data gathered right after the membrane was prepared, and there was no significant change in the slope of the ISE's response after two months.

Mass spectrometry

All experiments were performed on a Bruker micrOTOF-Q II mass spectrometer operating in negative ion mode to detect the ionophore-anion complex by electrospray ionization. Solutions were delivered directly to the spectrometer at a flow rate of ~3.0 µL min^{-1} with a Hamilton Unimetrics 250 µL syringe. The spray voltage was maintained at 2.8 kV. All solutions were prepared in methanol / chloroform 40/60 (%v/v). The concentration of the ionophores ranged from 0.5 µM to 10 µM. The ESI-MS isotopic patterns were resolved using the Molecular Weight Calculator by Mathew Monroe software version 6.49.

Results and Discussion

Potentiometric performance of clamshell ionophore in ISEs

Several membrane compositions (Table 1) were prepared to evaluate the potentiometric performance of the clamshell receptor as a neutral carrier for anions. Polyvinyl chloride (PVC) contains a slight excess of negative charge [11, 37] as a result of the manufacturing process. Consequently, PVC-based membranes show a weak cationic response.[13, 38, 39] For this reason, lipophilic cationic additives, such as TDMAC, have been generally added to the composition of neutral carrier-based ion-selective membranes to improve their permselectivity toward anions, neutralize ion–ionophore charges, and improve the Nernstian response.[37]

The membrane of composition I (Table 1) does not contain any of the ClS ionophore and accordingly gave a response consistent with the free energy of hydration of anions, which governs the phase transfer at the solution/membrane interface. Membrane I (Figure 2a) displayed a selectivity pattern that follows the typical Hofmeister series ($ClO_4^- > SCN^- > I^- >$ salicylate $> NO_3^- > Br^- > Cl^-$).[37]

The selective anionic response of the membranes only occurs after the addition of the ClS (Figure 2b). This behavior is a clear indication that the ionophore facilitates the selective transfer of anions from the solution to the membrane phase.

Generally, the concentration of charged lipophilic sites in the membrane has a significant impact on the overall characteristics of an ISE's response. Membranes with 25-160 mol% ratio of the ion-exchanger TDMAC relative to the ClS ionophore were prepared (Table 1), and the potentiometric response toward Cl^-, Br^-, NO_3^-, salicylate, I^-, SCN^-, and ClO_4^- evaluated.

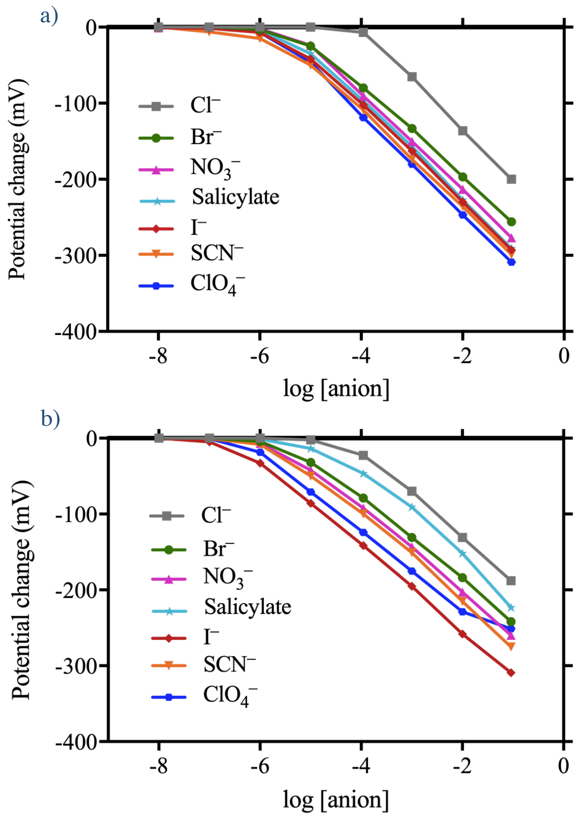

Figure 2. (a) Potentiometric response of TDMAC-based electrodes toward various anions in 0.5 M HEPES buffer, pH 7.4, with 0% ClS (membrane I). (b) Potentiometric response of electrodes composed of membrane IV (1 wt% ClS and 75 mol% TDMAC) toward various anions in 0.5 M HEPES buffer, pH 7.4 (membrane IV). Results shown are the average of triplicate measurements using three identical electrodes.

The electrodes demonstrated unstable responses at low mole ratios of TDMAC relative to clamshell and the residual charges of the membrane dominated the behavior of the electrodes. For instance, membrane II with 1 wt% ClS and 25 mol% TDMAC (Figure 3) and membrane VII with 2 wt% ClS and 25 mol% TDMAC displayed weak anionic response toward iodide of –26.6 mV/decade and –19.1 mV/decade over a linear range of 10 µM to 10 mM, respectively.

Presumably, higher concentrations of TDMAC are required to counterbalance the residual negative charges in the PVC membrane. On the other hand, increasing the concentration of TDMAC to stoichiometric (100 mol%) and super-stoichiometric (160 mol%) resulted in electrodes that returned unremarkable Hofmeister selectivity.

Use of intermediate levels of TDMAC (60-75 mol%) in membranes III (1 wt% ClS, 60 mol% TDMAC), IV (1%, 75%), IX (2%, 60%), X (2%, 75%), and XI (3%, 60%), resulted in improved performance with near-Nernstian response (Figure 3). Increasing the concentration of TDMAC from 60 to 75 mol% displayed an improvement in the performance of the electrode with 1 wt% ClS. Electrode IV with 1 wt% ClS and 75 mol% TDMAC demonstrated the optimum response toward iodide with a detection limit of 0.5 µM. The slope of the linear portion of the calibration plot of membrane IV is close to Nernstian (–58.2 mV/decade) over a linear range of 1 µM to 0.1 M (Figure 2b).

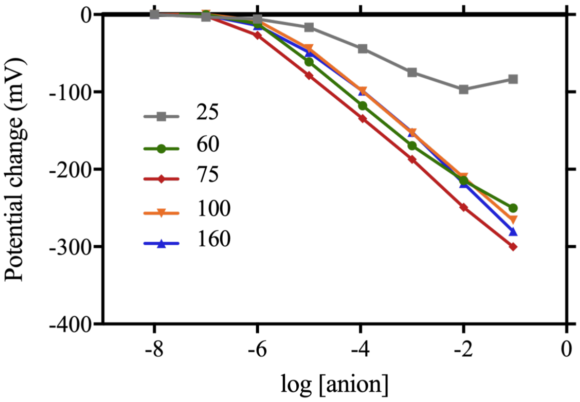

Figure 3. Iodide potentiometric response of ClS-based electrodes containing 1 wt% ionophore and different mole ratios of TDMAC relative to the ionophore (membranes II-VI). Results shown are the average of triplicate experiments.

The potentiometric response toward iodide was changed from –49.8 mV/decade for electrode III (1%, 60%) to –59.1 mV/decade for electrode IX with the same mole ratio of TDMAC relative to ClS but with 2 wt% ionophore over the linear range of 1 µM to 0.1 M and with a detection limit of 0.5 µM. Increasing the concentration of ClS to more than 2 wt% in electrode XI with the same mole ratio of TDMAC relative to ionophore (60 mol%) and 3 wt% ClS demonstrated a slope of -57.9 mV/decade over the linear range of 1 µM – 0.1 M with the same detection limit. In the comparison of electrodes IV and X, which contain 75 mol% of TDMAC and double the amount of the ionophore from 1 to 2 wt% of ClS, respectively, there was no significant change observed in the potentiometric performance towards iodide.

The selectivity coefficients were measured by the separate solutions method [40]. The ClS-based electrodes III, IV, and VIII-XI (Table 1) were found to exhibit good selectivity for iodide over major lipophilic anions. Figure 4 shows the selectivity patterns of various ClS-based membranes and the ion-exchanger based electrode I with no ionophore. Among ClS-based membranes, electrode IV exhibited excellent selectivity toward iodide over various anions with the following selectivity coefficients over Cl^- ($10^{-3.04}$), Br^- ($10^{-1.71}$), NO_3^- ($10^{-1.67}$), and SCN^- ($10^{-1.23}$). All membranes containing clamshell demonstrated improved selectivity toward iodide over salicylate among which electrode XI is the best for iodide (I^-) over salicylate with a selectivity coefficient of $10^{-2.13}$. Salicylate is an interfering anion in iodide analysis stemming from its high lipophilicity that promotes phase transfer from aqueous solution to the PVC membrane. However, the planarity of the clamshell structure and the size of its cavity do not favor complexing salicylate [13] in a 1:1 intramolecular sandwich or a 2:2 discrete intermolecular sandwich complex (*vide infra*). Contrary to the Hofmeister series, salicylate showed the lowest response, after chloride, in electrodes III, IX, and XI (Figures 2b and 4). Increasing the concentration of TDMAC to 75 mol% in membrane IV with 1 wt% ClS and membrane X with 2 wt% ClS worsen the selectivity of iodide over salicylate ($10^{-1.01}$ and $10^{-0.82}$, respectively). However, increasing the concentration of the ionophore from 1 to 3 wt% in ClS-based membranes with 60 mol% TDMAC (ISE III, IX, and XI) showed slight enhancement in detecting iodide over salicylate ($10^{-1.68}$, $10^{-1.81}$, and $10^{-2.13}$, respectively). This response is controlled by selective interaction between the ionophore and anions. Increasing the concentration of ClS from 1 wt% to 2 wt% with 60 mol% TDMAC (electrodes III and IX) and with 75 mol% TDMAC (electrodes IV and IX), worsen the selectivity of the ISEs towards iodide over perchlorate. However, increasing the CIS from 2 wt% to 3 wt% (electrode IX and XI respectively) had no significant effect on the selectivity of iodide over perchlorate. This selectivity trend is consistent with the observed for CNstar-based membranes when increasing the ionophore content from 1 wt% to 2 wt% (Figure S1).

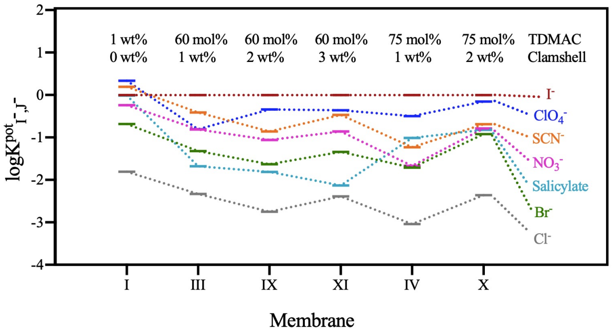

Figure 4. Potentiometric selectivity coefficients of clamshell- based electrodes containing different mole ratios of TDMAC (relative to ionophore) toward iodide over different anions in comparison to the ion-exchanger-based electrode I (1 wt % TDMAC).

The long-term stability of the ISEs was studied using membrane composition IV (1 wt% ClS and 75 mol% TDMAC). This membrane exhibits good long-term stability both in terms of overall potentiometric performance and selectivity toward iodide over a period of sixty days of use. The slope of the linear portion of the calibration plot of the electrode remained within the range of –58.4 mV/decade to –57.9 mV/decade over this period of time (Figure S2). Moreover, membranes stored dry at room temperature were stable for at least six months and performed as well as the fresh ones.

Ionophore Selectivity by Electrospray Ionization Mass Spectrometry

The clamshell has an architecture that could allow formation of two different elementary types of complexes with anions. The relative amount of these complexes will depend on the identity of the anion and can be of a 1:1 intramolecular sandwich complex (Figure 5a, b) and a 2:2 discrete intermolecular sandwich complex (Figure 5c-e). There are several studies that have successfully utilized mass spectrometry to analyze ion-ionophore complexation, characterize non-covalent complexes, and explain the behavior of ISEs.[41-45] To correlate the potentiometric selectivity of ISEs to the binding preferences of the clamshell and the parent CNstar, the complexation of these two ionophores was investigated with ESI-MS.

The selective gas-phase complexation of CNstar, the parent macrocycle, was investigated by ESI-MS experiments (Figure 6). In a first set of experiment, the solution containing an excess of the ionophore with a 10:1 ratio of CNstar to anions was studied by ESI-MS. We observed 1:1 complexes between CNstar and Cl^-, NO_3^-, Br^-, ClO_4^-, I^-, and salicylate with intensities of less than 4%. Complexes of higher 3:2 stoichiometries $[3CNstar + 2ClO_4]^{2-}$, $[3CNstar + I + ClO_4]^{2-}$, and $[3CNstar + 2I]^{2-}$ were also observed with a relative abundance of 21.7%, 9.7%, and 1.2%, respectively. The highest peak intensity was at *m/z* of 1931.9, which corresponds to the 2:1 complex $[2CNstar + ClO_4]^-$. The peak that appeared at *m/z* of 1945.6 corresponds to the 4:2 heterocomplex of $[4CNstar + I + ClO_4]^{2-}$ followed by the 2:1 complex of $[2CNstar + I]^-$ at *m/z* of 1959.4, both with relative intensities of less than 10%. The peak observed at *m/z* of 2848.2 was attributed to a 3:1 complex $[3CNstar + ClO_4]^-$.

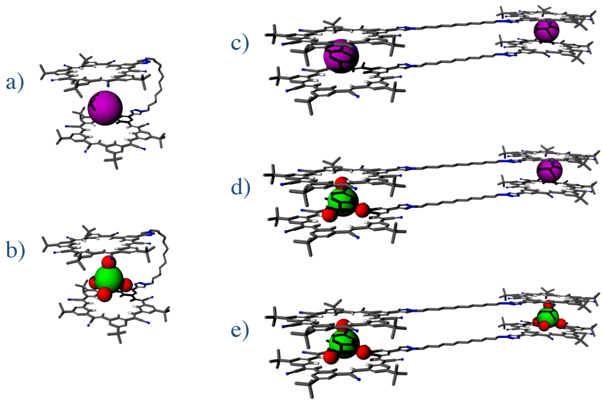

Figure 5. Schematics of complex formations of the clamshell with iodide and perchlorate. 1:1 intramolecular sandwich complexation of ClS with (a) iodide and (b) perchlorate. 2:2 discrete intermolecular sandwich complexation of two molecules of ClS with (c) two iodide anions, (d) one iodide and one perchlorate, and (e) two perchlorate anions.

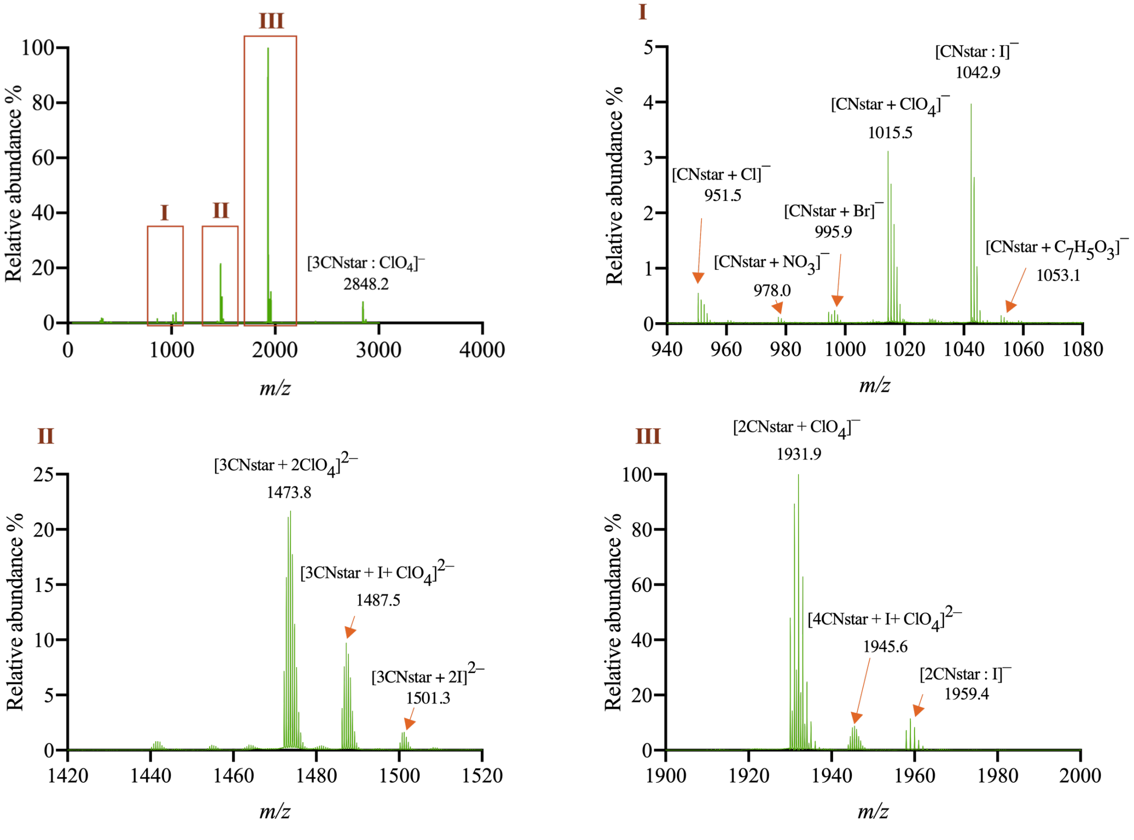

Figure 6. ESI-MS spectra of a solution of 5 µM CNstar, 500 nM NaCl, NaSCN, $NaNO_3$, NaBr, $NaClO_4$, NaI, and $NaC_7H_5O_3$ (sodium salicylate) in MeOH/$CHCl_3$ 40/60 v/v. On the top left, the overview of ESI-MS spectra is presented, and panels I, II, and III are zoomed-in spectra of the same plot. Each peak represents the complex of the CNstar and one or more anions as follows: m/z =951.5, $[CNstar + Cl]^-$; m/z= 978.0, $[CNstar + NO_3]^-$; m/z=995.9, $[CNstar + Br]^-$; m/z=1015.5, $[CNstar + ClO_4]^-$; m/z=1042.9, $[CNstar : I]^-$; m/z=1053.1 $[CNstar + C_7H_5O_3]^-$; m/z=1473.8, $[3CNstar + 2ClO_4]^{2-}$; m/z=1487.5, $[3CNstar + I+ ClO_4]^{2-}$; m/z=1501.3, $[3CNstar + 2I]^{2-}$; m/z=1931.9, $[2CNstar + ClO_4]^-$; m/z= 1945.6, $[4CNstar + I+ ClO_4]^{2-}$; m/z=1959.4, $[2CNstar : I]^-$; and m/z=2848.2, $[3CNstar : ClO_4]^-$.

These results are in agreement with the previous potentiometric study, which reported the selectivity of CNstar toward perchlorate.[13]

The observation of the higher order complexes with the parent CNstar macrocycle is consistent with our recent observations.[46, 47] In those cases, we observed crystal structures of the 2:2 and 4:3 CNstar : anion complexes as well as evidence for 3:2 and 3:3 species in solution by NMR spectroscopy and ESI-MS analyses. In these cases, however, the complexes were formed with bisulfate (HSO_4^-) and dihydrogen phosphate ($H_2PO_4^-$) hydroxyanions that are capable of donating and accepting hydrogen bonds between anions [48, 49]. The fact that there are no additional stabilizing interactions between the anions is consistent with the highly stabilizing environment of the macrocycles. With two anions sitting inside the pair of stacked cyanostar macrocycles, they will induce some electrostatic repulsions between them. Presumably, this repulsion forces the two anions to reside as far away from each other while still being stabilized by the macrocycles. It should be noted that complexes between multiple aprotic anions and charge-neutral receptors have been seen in a few cases.[50]

The ratio and concentration of the ionophore and anions also play a role in the type of host-guest complex that forms. For instance, in separate ESI-MS experiments (Figure S3), in the presence of 5 nM of the parent CNstar, and a massive excess of anion, e.g., 500 nM $NaClO_4$ and 500 nM NaI (ratio of ionophore to anions is 1: 100), the population of the 2:1 sandwich complex with perchlorate decreased dramatically, while that of the 1:1 iodide complex increased. However, after increasing the concentration of the CNstar to more than 100 nM in the presence of the same concentration of 500 nM anions, the complex formation preference shifted toward the 2:1 perchlorate complex (Figure 6). This behavior is consistent with the theory of complexation that lower concentrations of ionophore favor formation of the 1:1 complex. However, by increasing the concentration of CNstar to 5 μM in the presence of 500 nM anions (changing the ratio of ionophore to anions to be 10: 1), there is sufficient ionophore present in the solution to favor the 2:1 complex of CNstar : perchlorate, which was observed as the dominant complex in the ESI-MS study (Figure 6). Additionally, our prior work with ISEs based on the parent CNstar, demonstrated that the selectivity of the electrode for perchlorate over iodide improved when the concentration of CNstar in the membrane increased from 1 to 2 wt% (electrodes VII and IV, respectively, in reference 13).[13]

The potentiometric selectivity of the clamshell electrodes towards iodide and perchlorate were compared to the relative intensities of the ESI-MS peaks corresponding to the anion-ionophore complexes. The mass spectra were obtained with negative ionization under conditions where the concentration of iodide and perchlorate were fixed at 50 nM, while the clamshell's concentration was changed from 0.5 μM to 5 μM and to 10 μM; corresponding to 10, 100, and 200-fold excess of ionophore, respectively (Table 2).

Table 2. Comparison of complex formation of different concentrations of clamshell in the presence of fixed concentration of iodide and perchlorate based on the ESI-MS spectra.

[ClS]	**[ClO_4^-] and [I^-]**	**Ratio of ClS to each anion**	**Anion**	**Relative abundance% of 1:1 intramolecular**	**Relative abundance% of 2:2 intermolecular**	**Total relative abundance%**
0.5 μM	50 nM	10:1	ClO_4^-	82	0	82
			I^-	54	46	100
5 μM	50 nM	100:1	ClO_4^-	33	39	72
			I^-	53	47	100
10 μM	50 nM	200:1	ClO_4^-	42	58	100
			I^-	52	37	89

In the first set of experiments, where the concentration of ClS was 0.5 μM, the resulting spectra showed a dominant peak (100%) at *m/z* of 2147.6 that was attributed to an overlap between the 1:1 and 2:2 complexes $[ClS + I]^-$ and $[2ClS + 2I]^{2-}$, respectively. Resolving this peak by considering the isotopic pattern of each complex resulted in relative abundances of 54% and 46% for 1:1 and 2:2 stoichiometries, respectively. The next highest peak at *m/z* of 2120.1 was assigned to the 1:1 complex $[ClS + ClO_4]^-$ with a relative abundance of 82%, while there was no significant peak observed for the 2:2 intermolecular complex of ClS and ClO_4^- (Figure S4). Iodide and perchlorate anions compete with one another to fill the cavities of the CNstar macrocycles in the clamshell, however, the peak for the iodide complexes is modestly larger than the perchlorate one indicating higher relative abundance of the clamshell and iodide complexes ($[2ClS + 2I]^{2-}$ and $[ClS + I]^-$) compared to the complexes with perchlorate. This preference is consistent with the potentiometric selectivity pattern of the ClS-based electrodes.

By increasing the concentration of clamshell by one order of magnitude to 5 μM, the most abundant peak observed at *m/z* of 2147.6 was again attributed to the 1:1 and 2:2 multimers $[ClS + I]^-$ and $[2ClS + 2I]^{2-}$ with relative abundances of 53% and 47%, respectively. Another relatively significant peak at *m/z* of 2120.1 (total of 72% relative abundance) was attributed to the $[ClS + ClO_4]^-$ (33% relative abundance) and $[2ClS + 2ClO_4]^{2-}$ (39% relative abundance) complexes (Figure 7a). The results indicate that with excess clamshell, perchlorate forms a 2:2 intermolecular sandwich complex of two molecules of ClS with two perchlorates (Figure 5e) in addition to the 1:1 intramolecular sandwich complex (Figure 5b).

Doubling the concentration of ionophore to 10 μM, resulted in a change in the dominant peak in the ESI-MS spectra from iodide to perchlorate, while the relative contribution of the 1:1 and 2:2 ClS and ClO_4^- complexes changing to 42% and 58%, respectively (Figure S5).

We observe that the clamshell can form complexes with a mixture of anions in three different arrangements; intramolecular 1:1 (Figure 5a, b) and intermolecular 2:2 sandwich complexes with either the same anions (homo) or two different anions (hetero) (Figure 5c-e). The clamshell's heterocomplex with

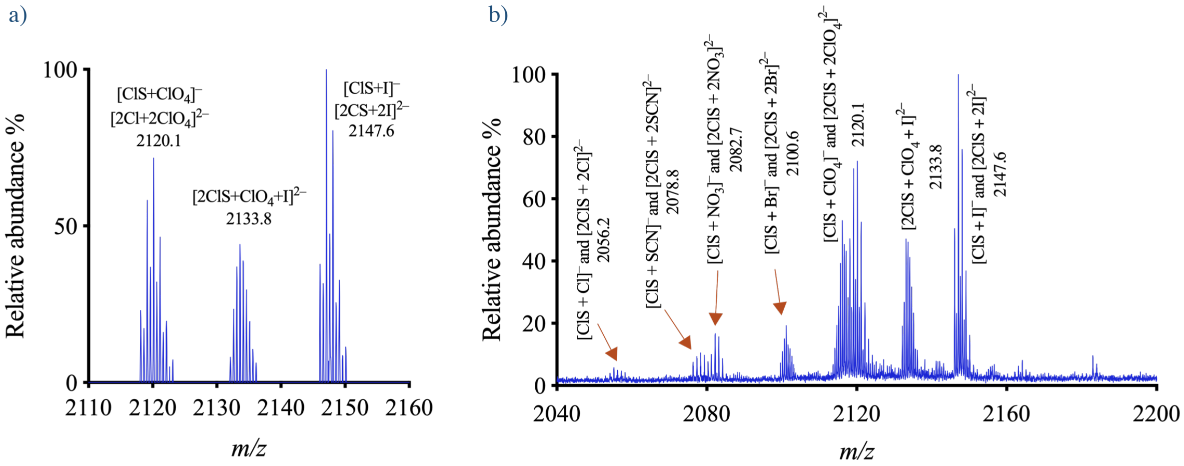

Figure 7. (a) ESI-MS negative mode spectra of the solution of 5 µM clamshell, 50 nM $NaClO_4$ and 50 nM NaI in $MeOH/CHCl_3$ 40/60 v/v. (b) ESI-MS spectra of the solution of 5 µM clamshell, with 100 nM each of NaCl, NaSCN, $NaNO_3$, NaBr, $NaClO_4$, NaI, and $NaC_7H_5O_3$ (sodium salicylate). Each labelled peak represents the complex of the clamshell and one or more anions; m/z=2056.2, $[ClS + Cl]^-$ and $[2ClS + 2Cl]^{2-}$, m/z=2078.8, $[ClS + SCN]^-$ and $[2ClS + 2SCN]^{2-}$, m/z=2082.7, $[ClS + NO_3]^-$ and $[2ClS + 2NO_3]^{2-}$, m/z = 2100.6, $[ClS + Br]^-$ and $[2ClS + 2Br]^{2-}$, m/z=2120.1, $[ClS + ClO_4]^-$ and $[2ClS + 2ClO_4]^{2-}$, m/z=2133.8, $[2ClS + ClO_4 + I]^{2-}$, m/z=2147.6, $[ClS + I]^-$ and $[2ClS + 2I]^{2-}$.

both iodide and perchlorate $[2ClS + I + ClO_4]^{2-}$ was observed, at *m/z* of 2133.8, in all experiment sets with a relative abundance of ~44-50% (Figure 7a, S3, S4). This interesting finding indicates that clamshell has the ability to complex two different anions at the same time and provides additional evidence for formation of the 2:2 intermolecular sandwich complex.

In addition to iodide and perchlorate, the binding behavior of the clamshell in the presence of Cl^-, Br^-, NO_3^-, salicylate, and SCN^- anions was explored using ESI-MS (Figure 7b, S6). The spectra reflect a solvent-free environment in which both electronic effects and size-selectivity influence complex formation and stabilization in the gas-phase.[29] All anions (Cl^-, Br^-, NO_3^-, salicylate, SCN^-, I^-, and ClO_4^-) demonstrated various complexation intensities with clamshell in both 1:1 and 2:2 stoichiometries. The most abundant peak in ESI-MS spectra in Figure 7, was assigned to the iodide complexes with 100% relative abundance followed by the perchlorate complexes with 72% relative intensity. The next significant peak corresponded to the 2:2 heterocomplex between the clamshell, iodide and perchlorate. The peaks for the complexes of Cl^-, Br^-, NO_3^-, and SCN^- anions were all less than 20% in relative abundance. Consistent with the observed potentiometric selectivity, the three highest peaks showed preferential complexation with iodide and perchlorate. There was no peak at a *m/z* of 2157.8 corresponding to the salicylate complex. Salicylate also showed a low response in the potentiometric study. Although lipophilic and being located before iodide in the Hofmeister series, the large size of the salicylate ion prohibited its stabilization within the clamshell cavity.[51]

Conclusion

A new CH hydrogen bonding macrocycle, clamshell, was used as an ionophore in developing anion-selective electrodes. It has been previously reported that cyanostar allows sandwich complexation with perchlorate as a 2:1 species. Herein, we demonstrate that clamshell, which is composed of two cyanostar macrocycles bridged with a 12-carbon chain, displays an altered selectivity pattern and different type of complex formation with anions. The optimized ClS-based electrode demonstrated *anti*-Hofmeister selectivity toward iodide with a slope of –58.2 mV/decade over a linear range of 1 µM to 0.1 M and a detection limit of 0.5 µM. The results of the potentiometric selectivity of electrodes prepared with the clamshell and cyanostar ionophores were in agreement with complexation data obtained with ESI-MS. Based on the ESI-MS study, the clamshell could also form 2:2 intermolecular sandwich complexes as well as 1:1 intramolecular sandwich complexes with anions. The clamshell also demonstrated the ability to form a 2:2-type heterocomplex. These findings provide a basis for modulating the selectivity of ion-selective electrodes by transforming bimolecular complexation events into unimolecular ones simply by connecting two receptors together into a single clamshell architecture.

ASSOCIATED CONTENT

Supporting Information

Lifetime potentiometric measurements; ESI-MS spectrum of a solution of CNstar, $NaClO_4$, and NaI; ESI-MS spectrum of solution of clamshell, $NaClO_4$, and NaI; ESI-MS spectrum of solution of clamshell, NaCl, NaSCN, $NaNO_3$, NaBr, $NaClO_4$, NaI, and $NaC_7H_5O_3$.

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Author Contributions

The authors declare no competing financial interest. EMF synthesized the ionophore. All other laboratory experiments were performed by EZ. All authors contributed to the writing of the manuscript and have given approval to the final version of the manuscript.

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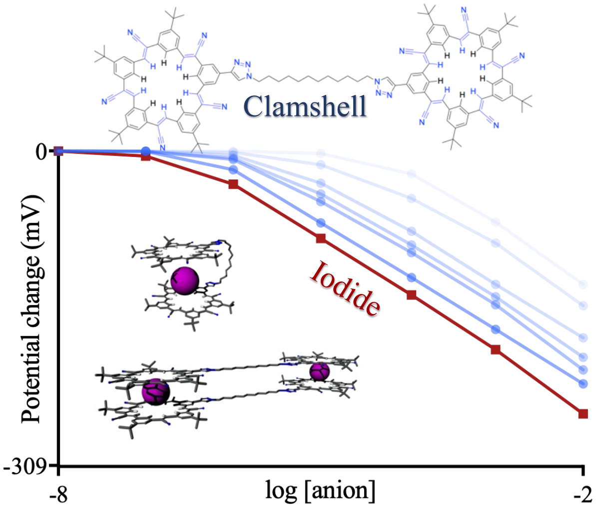
Clamshell
0
-309
Potential change (mV)
Iodide
-8
log [anion]
-2