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Structure-Selective Recognition by Voltammetry: Enantiomeric Determination of Amines Using Azophenolic Crowns in Aprotic Solvent

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The enantiomeric recognition of amines by voltammetry using electroactive macrocyclic molecules, nitroazophenolic crown ethers, is reported. The oxidation potential of the nitroazophenol moiety in nitroazophenols with 18-crown-6 sensitively depends on the structure of alkyl amines. Based on this phenomenon, enantiomeric amines and even the quantitative assay of the *R*/*S* ratio in enantiomeric mixtures can be selectively recognized by using 18-crown-6 azophenol (3-H) with chiral centers. In the case of phenylglycinol, the association constants (*K*) of 3-H for the *R* and *S* forms have an *R*/*S* value of 3.5. The peak potential of the *R* form in square-wave voltammograms reproducibly differs from that of the *S* form by 32 mV, within which the peak potential linearly varies with the enantiomeric ratio. Free energy perturbation and molecular dynamics simulation provide deeper understanding of the enantiomeric recognition in this system. The theoretical analysis indicates that the free energy difference between diastereomeric complexes agrees well with the experimental results, and the $\pi-\pi$ or charge−charge interaction plays a key role in enantiomeric recognition.

Voltammetry is a well-known analytical technique that is sensitive, cost-effective, and convenient. However, there are a few critical drawbacks such as a lack of structural selectivity and limited applicability to electroactive samples. There have been attempts to take advantage of voltammetry to selectively recognize and quantify electroinactive species by overcoming these drawbacks. The use of some redox-active calixarenes was reported to recognize ammonia and primary, secondary, and tertiary aliphatic amines in aprotic media.[1−5] However, there are a few reports on the voltammetric recognition of stereoisomers including cis−trans isomers[6] and enantiomers.[7−10] These studies on enantiomeric selectivity provide very limited analytical information. Kuhn and Anson, for instance, did not go beyond voltammetric discriminative features, and no quantitative investigation was made.[7] Ryabov et al. relied on enzymatic specificity, which can be exclusively applied to the water-soluble substrates corresponding to the enzymes employed.[8] Abbott et al. utilized quaternary ammonium derivatives that required further improvement in quantitative performance.[9] Another paper showed that the D/L forms of phenylalanine could be enantioselectively analyzed by using polyaniline while the origin of the structural recognition ability was not investigated systematically.[10] Overall, structural investigations in depth have been rarely addressed on the basis of electrochemical techniques. In particular, there is no report on the quantitative discrimination of enantiomeric alkyl amines by voltammetry to our best knowledge.

The azophenolic crown ethers employed in this study consist of two parts, dinitroazophenol and crown ether. The dinitroazophenol group acts as an indicator, showing the presence of amines by causing drastic changes in color as well as electrochemical behavior. On the other hand, the crown ether ring provides a cavity-like structure accommodating complexation. **1**-H[11−17] and **3**-H[18] in Figure 1 are the derivatives of azophenolic

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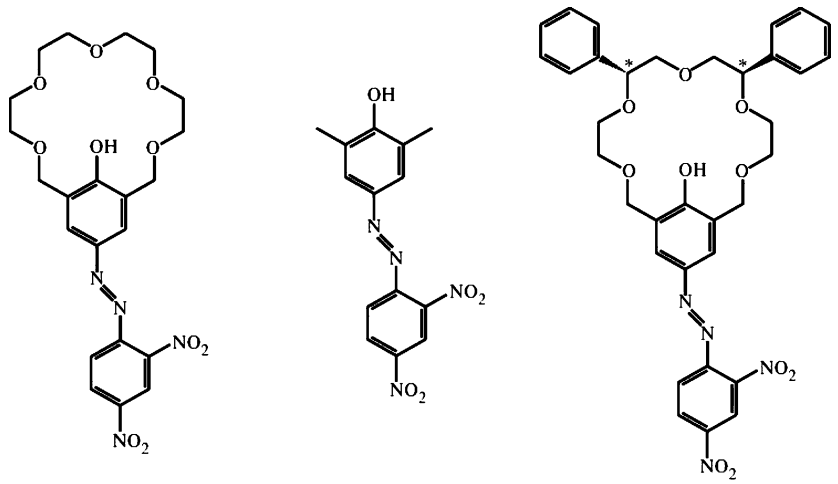

Figure 1. Structures of azophenolic acerands. **1**-H, **2**-H, and **3**-H denote crowned (2,4-dinitrophenylazo)phenol, (2,4-dinitrophenylazo)phenol, and chiral crowned (2,4-dinitrophenylazo)phenol, respectively.

crown ether and were reported to respond sensitively to the association with some metal ions and organic compounds, while **2**-H is a simple azophenol compound without a crown ring.

Besides experimental investigation, theoretical approaches can be systematically applied to a series of hosts by reproducing the experimental findings. This can help us to understand the features essential for the desired function of the host.[19] In this study, free energy perturbation (FEP)[20] and molecular dynamics (MD) simulations[21] were conducted to rationalize the enantiomeric discrimination mechanism of nitroazophenolic crown ether.

The goal of this report is to demonstrate the usefulness of voltammetry for the enantiomeric discrimination of various amines. In addition, a computational approach to elucidate the origin of the selective association of nitroazophenolic crown ethers with chiral centers is described.

EXPERIMENTAL SECTION

Synthesis and Reagents. "Crowned" 4-(2,4-dinitrophenylazo)-phenol (**1**-H), "uncrowned" 4-(2,4-dinitrophenylazo)phenol (**2**-H), and homochiral azophenolic crown ether (**3**-H) were synthesized according to the procedure in the literature.[22−24] Methylbenzylamine and electrochemical grade tetrabutylammonium perchlorate (TBAP) were used as received from Fluka. HPLC grade acetonitrile from Fisher was used as a solvent. Other reagents from Aldrich were used without further purification.

Electrochemistry. Electrochemical experiments were performed with a Windows-driven BAS100B/W electrochemical analyzer (Bioanalytical Systems, West Lafayette, IN) using a conventional three-electrode cell, with a glassy carbon working electrode, platinum wire counter electrode, and Ag/Ag^+ (0.01 M $AgNO_3$) reference electrode, separated from the solution by a Vycor plug. The potential of the reference electrode was confirmed and corrected by checking the redox potential of 1.0 mM ferrocene with the same electrochemical cell before every voltammetric experiment. The surface of the working electrode was polished with 0.3-μm alumina (Buehler, Lake Bluff, MN), rinsed with deionized water, and washed carefully with the solvent to be used. The supporting electrolyte was 0.1 M TBAP. The concentration of the acerands (**1**-H, **2**-H, **3**-H) was 0.50 mM, and an appropriate amount of the solution of an alkyl amine was added with a microsyringe. All experiments were carried out in a nitrogen atmosphere at room temperature.

RESULTS AND DISCUSSION

Voltammetric Responses in the Presence of Amines. In the voltammetric behavior of **1**-H, a single sharp peak emerges at ~1.2 V due to the oxidation of the phenol group of **1**-H. Both **2**-H and **3**-H produce the same voltammograms as **1**-H. This shows that neither the crown ether ring structure nor the electroinactive lariats influence the oxidation of nitroazophenol. Therefore, there is no factor affecting the electrochemical oxidation of **1**-H, **2**-H, and **3**-H except the characteristic interaction between the hosts and the guests. As a result of the incremental addition of an alkyl amine, a new oxidative wave appears and grows at a much less positive potential than that due to the host itself. This difference between the peak potentials (ΔE_p) of the two waves is a characteristic of the structural feature of alkyl amines.

Enantiomeric Recognition by 3-H. The host molecule employed in this study is **3**-H, which was reported as a chiral-selective indicator.[18,25] When a few colorless enantiomeric amines, **4**−**9** in Figure 2, were employed as guests, the enantiomeric recognizing capability of **3**-H could be verified by the spectrophotometric method, as shown in Supporting Information S-1.

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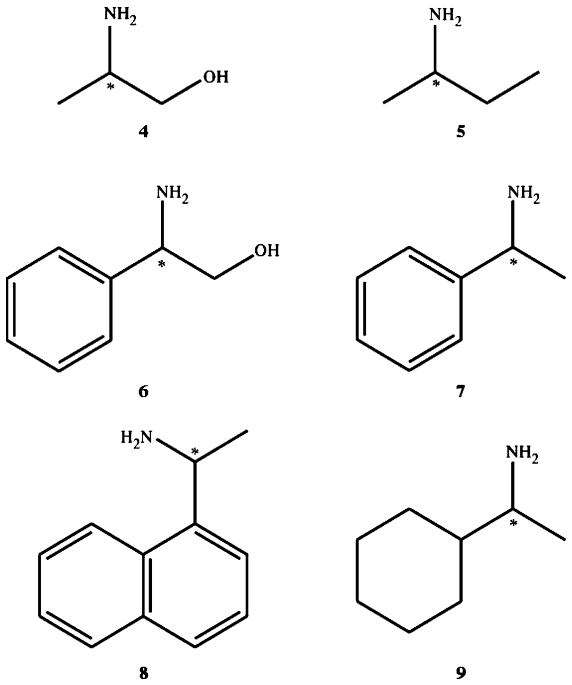

Figure 2. Structures of chiral amines investigated in this study.

The spectrophotometric results show that the large aromatic group and alcohol group at the end of the alkyl branch help the enantiomeric discrimination by **3**-H. With respect to the key factors that determine chiral selectivity, a theoretical approach can provide further insight. The enantiomeric recognition of amines using chiral acerands has been reported extensively by Naemura et al.[26−28] Based on CPK model studies, these reports pointed out that the major factor in enantiomeric recognition is steric hindrance. However, detailed studies such as FEP and MD simulations provide a deeper understanding of the host−guest complex formation. FEP simulations allow the calculation of free energy differences that can be directly compared with the experimental results. Since FEP simulations (see Supporting Information S-2) calculate the energy difference between the two states, the errors in the free energy difference estimation are much smaller than those from other methods that calculate the free energies of the two states separately and then obtain the difference by subtracting one from the other.[29]

The calculated FEP and spectrophotometrically determined values for the enantioselectivity of $\mathbf{3}^-$ are listed in Table 1. The calculated values are in excellent agreement with the experimental results. Only in the case of the $\mathbf{3}^-$−**5** complex, the (*R*)-**5** complex is slightly more stable in the experiment, whereas the (*S*)-**5** complex is more stable in the FEP calculation. The range of the free energy difference of the $\mathbf{3}^-$−**5** complex in the FEP calculation, however, is −0.3 to +0.5 kJ/mol. This means that $\mathbf{3}^-$ cannot discriminate between the enantiomers of **5** because the side chain of **5** is too small. Also, the diasteromeric complex of chiral pyridino crown ether and **5** have been shown in our previous report.[30] For the $\mathbf{3}^-$−**4** complex, the error bar of the experimental values lies outside the standard deviation of the computed result, but the error intervals are separated by only 0.5 kJ mol^{-1}, and (*R*)-**4** is proved to be more stable both by the experimental and simulation results.

Table 1. Calculated and Experimentally Determined Enantioselectivity of 3-H for 4−9[a]

guest	$\Delta\Delta G_{Exp}$[b]	$\Delta\Delta A_{FEP}$[c]	more stable enantiomer[d]
4	−0.9 ± 0.1	−1.8 ± 0.3	*R*
5	−0.2 ± 0.1	0.1 ± 0.4	*R*
6	−3.1 ± 0.1	−2.7 ± 0.8	*R*
7	1.8 ± 0.2	1.5 ± 0.3	*S*
8	2.4 ± 0.2	1.8 ± 0.6	*S*
9	0.2 ± 0.3	0.3 ± 0.3	*S*

[a] All units are in kJ mol^{-1}. [b] Values determined by spectrophotometric titration (Supporting Information S-1). [c] Free energy obtained by FEP with 500-ps sampling per window. [d] More stable enantiomer of the guest in the experiment.

The MD simulations, which are described in Supporting Information S-2, strongly suggest that the NH···O hydrogen bonding is involved in the host−guest complexation but it does not explain the enantiomeric recognition. The second interaction such as additional hydrogen bonding or the $\pi-\pi$ interaction determines the relative orientation between host and guest molecules. The third interaction such as $\pi-\pi$ or charge−charge is also a crucial factor for enantiomeric recognition. In contrast to the CPK model studies of Naemura et al., which suggest the steric hindrance as the major factor in enantiomeric discrimination, our detailed simulation analysis shows that the $\pi-\pi$ and charge−charge interactions are major factors in the enantiomeric discrimination of $\mathbf{3}^-$ toward chiral amine guest molecules.

Enantiomeric Determination of Amines by Voltammetry. The results described hitherto briefly state that ΔE_p depends on how strongly the amines modify the electronic configuration of the chromogenic dinitroazophenol group. The apparent correlation between K_R/K_S and $\Delta\lambda_{max}$ in Table 1 indicates that the respective isomers of an amine form complexes with **3**-H with different stabilities and affect the electronic configuration discriminatively. There are two properties of amines that play crucial roles in determining ΔE_p: the basicity and the structural feature of amines. Since the isomers are considered to have equal basicity, the influence of different basicities can be neglected. Instead, ΔE_p exclusively depends on the chiral-selective complexation between **3**-H and the enantiomers. Therefore, ΔE_p is suggested as a new indicator to recognize a specific enantiomer and quantify the ratio of enantiomers in a mixture.

Figure 3 a shows the square-wave voltammograms (SWV) of **3**-H in the presence of (*R*)-**6** and (*S*)-**6**. The difference between the oxidation potentials of the $\mathbf{3}^-$−H^+-(*R*)-**6** and $\mathbf{3}^-$−H^+-(*S*)-**6** complexes, $\Delta\Delta E_p$ (=$\Delta E_{p,(R)} - \Delta E_{p,(S)}$), is −44 mV. The $\Delta\Delta E_p$ data are reproducible in the successive measurements and are large enough to distinguish one from the other with a standard deviation less than 3 mV. Table 2 summarizes the voltammetric responses to the enantiomers of **4**−**9** by SWV. The E_p of an isomer that forms a more stable complex than the other is more positive,

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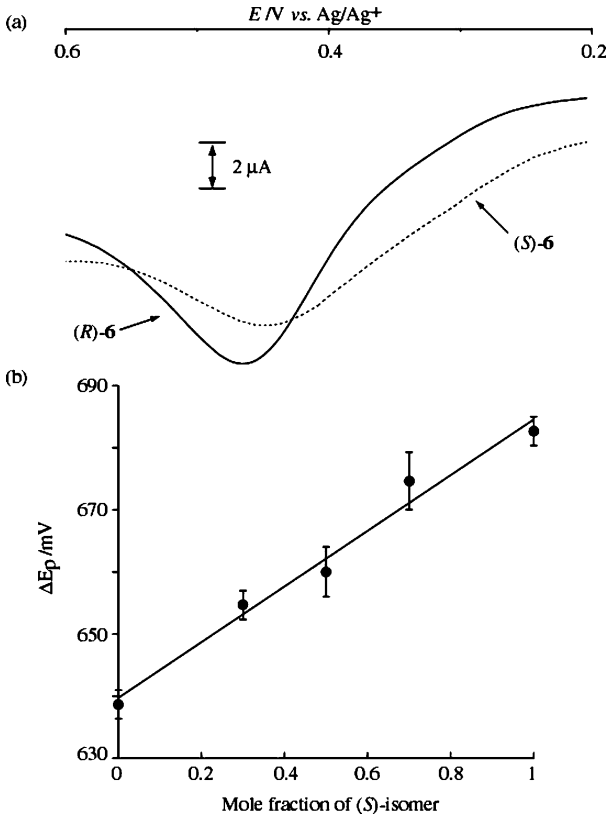

Figure 3. (a) Square-wave voltammograms of 0.5 mM **3**-H solution in the presence of 0.25 mM (*R*)-**6** (solid curve) and (*S*)-**6** (dotted curve). The step potential, amplitude, and frequency for SWV are 4 mV, 25 mV, and 30 Hz, respectively. (b) The relationship between E_p and mole fraction of *S* isomer ([(*S*)-**6**]/([(*R*)-**6**] + [(*S*)-**6**])) by SWV. [**3**-H] and [(*R*)-**6**] + [(*S*)-**6**] are equal, 0.5 mM. The brackets denote concentration.

Table 2. Shift in the Oxidation Potentials Due to the Enantiomeric Amines of 4−9

	ΔE_p[a] (mV)		
amine	*R*	*S*	$\Delta\Delta E_p$[b] (mV)
4	733 (±6)	776 (±0)	−43 (±6)
5	802 (±5)	806 (±6)	−4 (±6)
6	639 (±2)	683 (±2)	−44 (±3)
7	718 (±4)	693 (±2)	25 (±4)
8	716 (±4)	693 (±5)	23 (±6)
9	835 (±2)	837 (±2)	−2 (±3)

[a] All data are the averages and standard deviations of three independent measurements obtained by square-wave voltammetry wherein **3**-H was employed as a chiral selective host. [b] $\Delta\Delta E_p = \Delta E_{p,(R)} - \Delta E_{p,(S)}$.

corresponding to a smaller ΔE_p. This means that an amine with $K_R/K_S > 1$ gives a negative $\Delta\Delta E_p$ and vice versa. Table 2 indicates that **4** and **6** show $K_R/K_S > 1$ and a negative $\Delta\Delta E_p$, while **7** and **8** with $K_R/K_S < 1$ show a positive $\Delta\Delta E_p$. The enantiomers of **5** and **9** are not distinguished clearly, which is consistent with the results of $\Delta\lambda_{max}$ from absorption spectrophotometry.

Based on the same principle, the ratio of an enantiomeric mixture can be determined by voltammetry. The peak in the square-wave voltammograms moves continuously and proportionally as the ratio of (*S*)-**6** to (*R*)-**6** varies, as shown in Figure 3b. The results are reproducible and accurate enough to assess the enantiomeric ratio. This method has a few valuable advantages. For example, this procedure is much simpler and more cost-effective than optical methods or mass spectrometry such that only a small quantity of a sample is required for analysis with a microelectrode system such as a micromachined electrochemical cell.

CONCLUSIONS

The oxidation potential of nitroazophenols with a crown ether ring is sensitive for characterizing the complexation with amines. This phenomenon was successfully utilized for enantiomeric recognition. When dinitroazophenol with a chiral center, **3**-H, is exposed to the *R* isomer or *S* isomer of an amine in a solution, the potentials of the new oxidation peaks (E_p) are quite different from each other. E_p is closely related to the association constant (K), and the selective complexation between **3**-H and the enantiomers causes a potential shift that can be used for the voltammetric recognition of the enantiomeric mixture. Molecular dynamics simulations showed that the $\pi-\pi$ and charge−charge interactions are the dominant factors in enantiomeric discrimination. As demonstrated, the voltammetric method proposed in this report offers a promising opportunity for determining excess enantiomeric mixtures. As expected, better synthetic receptors in this method would lead to further progress toward an easier and faster enantiomeric recognition of organic compounds.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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