Molecular imprinting of antibiotic films for electroanalysis of the dopamine/ascorbate system

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Molecular films of a natural antibiotic lasalocid A have been cast on the surface of glassy carbon electrodes and used for the differentiation of electrochemical signals in the model system composed of dopamine and ascorbate. The best signal resolution is obtained at the films that were prepared by evaporating solvent from a chloroform solution of lasalocid that was equilibrated with an aqueous solution of dopamine. The improved selectivity of such films is ascribed to their enhanced hydrophobicity. The latter is hypothesized to be the result of lasalocid's compact cyclic conformation, which was imprinted in the solid films by the complexation with dopamine. When operated at a constant potential of 0.20 V, the lasalocid-based dopamine sensors are free of interferences from ascorbate and displayed low detection limits (~70 nM dopamine, S/N=3) in conjunction with fast response times (~0.4 s).

The modification of electrodes with permselective films is an important consideration in the development of selective electrochemical sensors. In this paper, we describe amperometric sensors based on electrodes coated with films of a new modifier lasalocid A. The selectivity of such modified electrodes was studied using a classical analytical system of dopamine and ascorbate. The selective determination of the neurotransmitter dopamine in the presence of interfering ascorbate is important in the study of communication between neurons, because abnormalities in dopamine concentration in the brain have been associated with many neurological complications. In order to minimize the interferences from ascorbate, the electrochemical sensors for dopamine have been covered with permselective coatings which provided a degree of charge-based discrimination between cationic dopamine and anionic ascorbate. Examples of such coatings include the ion-exchanging polymers and zeolites, electropolymerized films, self-assembled monolayers, and covalently modified carbon surfaces.

In the present study, we report a different approach to the signal differentiation in the dopamine/ascorbate system. It relies on molecular films of lasalocid A. Lasalocid A is a natural polycyclic antibiotic that is produced stereospecifically by the bacterium Streptomyces lasaliensis. It has been a subject of many biochemical and physicochemical investigations because of its ability to transport metal ions and amines across hydrophobic membranes. To the best of our knowledge the lasalocid A has not been used for the design of selective coatings for amperometric sensors.

We have selected lasalocid as an electrode modifier for two reasons. First, thin films of lasalocid are easily formed on solid substrates by evaporating solvent from an organic solution of lasalocid. Such films display good stability in aqueous solutions because lasalocid is practically insoluble in water. Second, lasalocid molecules display a considerable conformational freedom, which allows them to act as multidentate ligands for the complexation of metal ions and catecholamines including dopamine. Our hypothesis was that the molecular conformation of lasalocid complexed with dopamine would be preserved in solid films after the evaporation of a solvent. The rationale was that such electrode films would favor dopamine over ascorbate, thus providing a better resolution of voltammetric peaks due to the oxidation of these two analytes. This scenario is a variation on the theme of molecular imprinting of polymers, where monomers are oriented and polymerized around template molecules in order to provide recognition sites in a solid polymeric matrix.

The goal of the present work is to investigate the capability of different lasalocid films to differentiate the electrochemical signals of dopamine and ascorbate. The origin of signal differentiation is discussed using comparative spectroscopic and
Materials and Methods

Reagents and solutions
Lasalocid A sodium salt (LAS), 3-hydroxytyramine hydrochloride (dopamine, DA), ascorbic acid (AA), and chloroform were purchased from Sigma-Aldrich. Other chemicals, NaH$_2$PO$_4$•H$_2$O and NaOH were from Fisher. Aqueous solutions were prepared using deionized water (18 MΩ-cm) that was purified with a Barnstead NANOpure cartridge system.

The lasalocid solutions were prepared by dissolving the LAS in chloroform. The equilibration of chloroform solutions of LAS with aqueous solutions of DA (pH 7.40 phosphate buffer) was performed under argon by vigorous mixing using a magnetic stirrer.

The DA and AA solutions were always freshly prepared before each experiment using deoxygenated water. In all of the electrochemical experiments, the concentration of AA was 5x larger than that of DA. All experiments were performed at room temperature (21±1°C).

Electrochemical measurements
Cyclic voltammetry and flow injection amperometry were performed with the CH Instruments model 832 electrochemical detector. All experiments were performed in a conventional three-electrode system with a glassy carbon electrode (Bioanalytical Systems, Inc., BAS), platinum wire as the auxiliary electrode, and the Ag/AgCl/3MNaCl (BAS) reference electrode. The glassy carbon electrodes were polished prior to the experiments on sandpaper (3M Grain 2000) and metallographic cloth (Mark V Lab) with successively smaller particles (0.3 and 0.05-µm diameter) of alumina suspended in deionized water. After each polishing step, the slurry accumulated on the electrode surface was removed by a 20 s ultrasonication in deionized water.

The flow system consisted of a reservoir of carrier solution that was connected to a six-port injection valve (V-451, Upchurch Scientific, Inc.) with a sample loop of ~100 µL. The outlet of the valve was connected to a cell with Teflon tubing of 1.0-mm inner diameter. The modified electrode was positioned at the outlet of the tubing using a micromanipulator. The carrier solution was gravity fed at 5 mL min$^{-1}$.

The pH 7.40 phosphate buffer solution (0.10 M) served as a background electrolyte in all electrochemical experiments. The experiments were repeated at least three times and the means of measurements are presented with the standard deviations.

Spectroscopic measurements
The electronic spectra were recorded with a HP-8453 UV/visible diode array spectrophotometer using a quartz cuvette with a path length of 1.0 cm. Mass spectrometric analysis of the solutions was performed using the electrospray ionization-quadrupole ion trap (ESI-QIT) method and Finnigan LCQ Duo ion trap mass spectrometer. The spectrometer was controlled by Finnigan Xcalibur software.

Electrode modification
The glassy carbon electrodes were modified by casting 20.0 µL of a chloroform solution of lasalocid. Both the original lasalocid solutions and those that were equilibrated with dopamine were used. The chloroform was evaporated for 1 h under vacuum in order to form thin solid films of lasalocid on the surface of electrodes.

Results and Discussion
Spectroscopic analysis of lasalocid solutions
The lasalocid (Fig. 1) is soluble in hydrophobic media. Two types of lasalocid solutions were investigated for the modification of electrode surfaces: (1) a chloroform solution of sodium lasalocid (LAS solution), and (2) a chloroform solution of sodium lasalocid that was equilibrated with an aqueous solution of dopamine (LAS-DA solution). A series of the LAS-DA solutions was prepared by changing the molar ratio of dopamine to lasalocid, $n_{DA}/n_{LAS}$, and maintaining the sum of moles $n_{DA} + n_{LAS}$ constant in the system. After 24 h of equilibration, the UV-visible spectra of chloroform phases were recorded. A spectrum of the original LAS solution was collected as well.

Figure 2 shows that the electronic spectra contained a characteristic π→π* electronic absorption band of LAS at 310 nm. In addition, an extra absorption appeared at shorter wavelengths as the $n_{DA}/n_{LAS}$ ratio in the system increased. This extra absorption can be ascribed to DA, which absorbs light in the 260-290 nm range. Evidently, DA molecules were extracted from an aqueous solution into the chloroform solution of LAS. A blank experiment showed that no
extraction occurred in the absence of LAS in the chloroform. These results indicated that interactions of DA with LAS were the driving force behind the transfer of DA from water to the organic phase.

A more detailed analysis of lasalocid solutions was performed using ESI-QIT spectrometry. The mass spectrum of the 50 μM sodium lasalocid solution in chloroform contained only one major peak at \( m/z = 1225 \), which indicated that lasalocid existed as a dimer ([LAS]₂Na₂). Such dimeric species have been proposed in literature based on the NMR analyses\(^{19}\).

The peak of dimer diminished largely after the equilibration of the lasalocid solution with the dopamine solution. The mass spectrum of such equilibrated lasalocid solution displayed two additional peaks at \( m/z = 744 \) and 613. These peaks proved that lasalocid, after equilibration with a DA solution, existed in chloroform primarily in the form of monomeric species of the LAS-DA complex (FW = 744) and LAS-Na salt (FW, 613), both with a 1:1 stoichiometry.

Previous studies have demonstrated that a crystalline salt containing equimolar amounts of dopamine and lasalocid could be precipitated from a methylene chloride solution\(^{20}\). The computational model of the lasalocid-dopamine complex\(^{21}\), which agrees with X-ray analysis of the lasalocid-amino-(bromophenyl)ethane complex\(^{20}\), has predicted a structure in which the aromatic rings of dopamine and lasalocid overlap, and the ammonium group of dopamine is docked to the basket formed by the oxygen atoms of lasalocid. The contour of such a complex is determined by the pseudo-macrocyclic conformation of the lasalocid. As a result, the complex assumes a shape of a cup with polar orifice and interior, and a hydrophobic outside surface\(^{22}\).

**Voltammetry of DA and AA at bare and lasalocid film electrodes**

The electrooxidation of dopamine (DA) and ascorbic acid (AA) involves the loss of two electrons and protons to produce dopamine-o-quinone and dehydroascorbate, respectively. Figure 3 shows that at bare glassy carbon electrodes, anodic peak potentials of DA and AA overlap preventing a reliable electrochemical determination of DA in the presence of AA. In order to separate the voltammetric peaks of DA and AA, the electrodes were modified with lasalocid films. Two types of surface films were investigated: (1) the LAS films that were prepared using the LAS solutions, and (2) the LAS-DA films cast from the LAS-DA solutions.
In a background electrolyte solution, the cyclic voltammograms at the LAS and LAS-DA film electrodes were featureless (Fig. 4, curves a\textsubscript{b} and b\textsubscript{a}, respectively). This indicated that lasalocid was electrochemically silent in the selected potential window. Furthermore, a voltammogram recorded at a LAS-DA film electrode showed no current peaks due to the redox of dopamine. Apparently, the concentration of DA in the LAS-DA film was below the detection limit, or, more probably, the dopamine leached quickly from the film into the aqueous background electrolyte solution.

Figure 4 was split into two panels to show voltammograms recorded at film electrodes in solutions of dopamine (Fig. 4A) and ascorbic acid (Fig. 4B). At the LAS film electrodes (curves a), the anodic peak potentials of DA and AA were shifted toward potentials that were more positive by -200 and -500 mV, respectively, than those recorded at a control bare electrode (curves c). At the LAS-DA film electrodes (curves b), the anodic peak of DA was shifted by -250 mV, while the peak due to the redox of AA was shifted entirely outside the selected potential window. Such changes in peak potentials reflected varying degrees of kinetic inhibition of the overall electrode processes of DA and AA. This resulted in a strong differentiation of DA and AA voltammograms recorded at electrodes coated with lasalocid films.

**Interpretation of signal separation in the DA/AA system at film electrodes**

Two possible scenarios were considered in order to explain the separation of anodic peaks of DA and AA at the lasalocid-coated electrodes. The first scenario involved a charge-based molecular recognition. In the pH 7.40 buffer solution, the dopamine (pK\textsubscript{a}=8.92) is a cation and ascorbic acid (pK\textsubscript{a}=4.10) exists as an anion. Thus, the permselectivity of the surface films could arise from deprotonated carboxylic groups of lasalocid (pK\textsubscript{a}=3.70), which can attract cations and repel anions. However, this explanation was challenged by the apparent lack of ascorbate repulsion by the LAS film. Figure 4B shows that, instead of being smaller, the peak current of ascorbate at a LAS film electrode (curve a) was slightly larger than that recorded at a control bare electrode (curve c). Apparently, the complexation of LAS carboxylic groups with Na\textsuperscript{+} ions\textsuperscript{2} restricted their differentiating electrostatic interactions with ascorbate C\textsubscript{6}H\textsubscript{5}O\textsubscript{3}H\textsuperscript{-} and dopamine C\textsubscript{6}H\textsubscript{4}OHCOO\textsuperscript{-} ions.

The alternative explanation assumes that a separation of DA and AA voltammograms is caused by the hydrophobicity of lasalocid films. The film hydrophobicity originates from a tendency of lasalocid molecules to adopt a pseudo-macrocyclic conformation with a hydrophobic outside surface. Thus, one can assume that voltammograms of DA and AA separate because the oxidation of hydrophilic AA at a hydrophobic film electrode requires more overpotential than the oxidation of a relatively hydrophobic DA.

Accordingly, a larger hydrophobicity of LAS-DA film than that of LAS film can be invoked to explain a better separation of anodic peaks of DA and AA at a
LAS-DA film electrode (Figure 4, curves b) than at a LAS film electrode (Fig. 4, curves a). Preliminary results of contact angle measurements appear to support the notion that surface of LAS-DA films is more hydrophobic than that of LAS films. Consistent with the spectroscopic data, the lasalocid exists predominantly in the form of monomeric species in LAS-DA solutions, and as a dimer in LAS solutions. One can postulate, in line with the theoretical conformational analysis of lasalocid, that the monomeric species acquire more compact cyclic form than the sandwich-type dimer species. We hypothesize that such a compact hydrophobic conformation of monomeric lasalocid remains imprinted in the solid state and introduces a higher degree of hydrophobicity to the LAS-DA films.

Optimization of LAS-DA films for electroanalysis of the DA/AA system

The ability of the LAS-DA film electrodes to resolve electrochemical signals of DA and AA depended on the equilibration time and concentrations of DA and LAS solutions that were used in the film preparation. Figure 5 shows the influence of these parameters on the separation of DA and AA voltammograms. Because some of the voltammograms displayed a current plateau instead of a current peak, the separation of the voltammograms was expressed as the $\Delta E_{1/2}$, which was a difference in either the half-peak or half-wave potentials of AA and DA. The largest $\Delta E_{1/2}$ and, thus, the best selectivity was obtained with the LAS-DA films that were prepared from chloroform solutions of 1.0 mM lasalocid, which were equilibrated with 2.0 mM dopamine solutions for 24 h. A casting of 20.0 μL of such lasalocid solution on the electrode resulted in a surface film containing 1.6x10^{-7} mol of lasalocid per cm^2. Such film electrodes were used to record cyclic voltammograms shown in Fig.4. More concentrated chloroform solutions of lasalocid (>1 mM) yielded thicker surface films. Thick lasalocid films acted as efficient diffusion barriers for both the DA and AA and, consequently, caused a comparable shift in anodic peaks of DA and AA preventing a better signal resolution.

In order to simplify the film preparation, methanol was used as a solvent because both the lasalocid and dopamine are soluble in methanol. However, a substitution of chloroform with methanol as a casting solvent resulted in electrode films that displayed lower signal differentiation between the dopamine and ascorbate. Such a behavior, still under investigation, revealed the importance of an aprotic nonpolar medium for structuring lasalocid films on the surface of electrodes.

Analytical performance of the film electrodes

Flow injection analysis (FIA) was used to examine the analytical performance of the film electrodes. In order to compare the selectivity of the film electrodes toward the dopamine in the presence of ascorbic acid, two solutions were injected in a flow system: (a) 3.0 μM DA, and (b) 3.0 μM DA + 15.0 μM AA. The selective electrode should respond to the injections of solutions a and b by generating current peaks of the same height.

Figure 6 shows a set of FIA peaks recorded at a constant potential of 0.20 V at the control bare glassy carbon, LAS film, and LAS-DA film electrodes. In agreement with the voltammetric studies, the bare electrode showed no selectivity, the LAS film electrode displayed an improved selectivity, and the LAS-DA film electrode demonstrated the best selectivity as indicated by the absence of AA interference in the determination of DA. The LAS-DA film electrode did not show any deleterious memory effects due to the DA preconcentration in the surface film as illustrated by the fast return of current to the baseline. In addition, under the experimental conditions used, the electrode showed no interference arising from possible homogeneous catalytic oxidation of AA by oxidized DA.
Compact cyclic conformation was imprinted in the films by evaporating solvent from solutions containing the lasalocid-dopamine complex. Such films were hydrophobic enough to provide a good resolution of electrochemical signals of hydrophilic ascorbate and a relatively hydrophobic dopamine. An aprotic nonpolar casting solvent was required for structuring of selective surface films of lasalocid.

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References