Electrochemically Stimulated Adenosine 5′-Triphosphate (ATP) Release through Redox Switching of Conducting Polypyrrole Films and Bilayers

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Received June 29, 1995. Revised Manuscript Received October 26, 1995[⊗]

Electrochemically stimulated adenosine 5′-triphosphate (ATP) release from poly(pyrrole adenosine 5′-triphosphate) (PP-ATP) was investigated using cyclic voltammetry. Two separate redox processes at $E_{1/2} = -0.4$ and 0.0 V were clearly elucidated by switching the film in polyelectrolyte solutions and found to correlate with cation and anion dominant transport, respectively. This voltammetric method allows the dominant mobile ionic species to be determined as redox processes associated with the movement of the polyelectrolyte counterion through the film are observed, while redox processes associated with transport of ions having the same charge as the polyelectrolyte are repressed. As such, the more anodic redox process results in ATP release. Spontaneous ATP exchange in a supporting electrolyte was examined at open circuit, revealing that less than 10% of the electrochemically releasable ATP is spontaneously released after 10 days. The ATP release concept was extended to bilayers consisting of PP-ATP as inner layers and poly(*N*-methylpyrrole) (PNMP) with various dopant anions as outer layers. PP-ATP:poly(*N*-methylpyrrole chloride) (PP-ATP:PNMP-Cl) bilayers with varied thicknesses of inner and outer films were constructed to examine electrochemically stimulated ATP release during reduction of PP-ATP. PP-ATP:PNMP/heparin (PP-ATP:PNMP/HPN) bilayers with varied outer film thicknesses, which are potentially biocompatible, were also prepared and their ATP release properties were compared. It was found that the rate of electrochemical ATP release can be controlled by adjusting each film thickness with minimal change of ionic strength of the switching medium.

Introduction

The electrochemical switching of conducting polymers is accompanied by ion movement to maintain charge neutrality with the identity of the mobile species, and direction of ion flux, controlled by polymer−ion interactions.[1] Conducting polymers having immobile high molecular weight or multianionic dopant species (e.g., polyelectrolytes) incorporated during electropolymerization exhibit cation-dominated transport. Smaller monoanionic dopants tend to be more mobile and can be exchanged with anions in the bathing electrolyte solutions.[1d,e]

The application of these properties to ionic drug and biomolecule release system, such as glutamate and protonated dopamine release from polypyrrole (PP) and poly(*N*-methylpyrrole)/poly(styrenesulfonate) (PNMP/PSS), was originally reported by Miller et al.[2] We have recently reported the electrochemically stimulated release of ATP from PP-ATP films.[3] In that study, we found that while ATP is not significantly spontaneously exchanged with small anions in bathing electrolyte solutions over a period of one day, an appreciable amount (ca. 80%) of the ATP dopants can be released in a few minutes during PP reduction. Importantly, the ATP ions were found to be inert toward hydrolytic cleavage during both electropolymerization and potential driven release, suggesting that PP-ATP electrodes may be useful as ATP supply devices. ATP is a critically important component to many biological functions. In addition to its well-known role in biological energy storage and conversion, it plays an important role in cardiovascular systems. Although the mechanism of the electrophysiological action of ATP on the mammalion heart is not fully understood, its clinical uses have been explored since the 1940s.[4] Presently in Europe, ATP is routinely used for treatment of acute paroxysmal supraventricular tachycardia.

Conducting and electroactive polymer modified bilayer electrodes, exhibiting cation- and anion-dominant transport behavior at separate potentials (potential dependent dual ion transport), have been introduced by our group.[5] In our work, studies of bilayers, consisting of low redox potential conducting polymer inner films (e.g., PP/PSS) and high redox potential electroactive polymer outer films (e.g., PNMP-Cl, poly(vinylfer-

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1995.

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rocene)), have shown that the redox switching and ion transport of the inner films are not hampered by the presence of the outer films and that the amount of each ionic species incorporated or released is related to the individual film composition. This bilayer design allows the redox chemistry of the outer film to be studied using the inner film as an extension of the conducting electrode. The inversely ordered film structure (high redox potential inner film and low redox potential outer film) was avoided since rectification behavior occurs.[6,7]

In this paper, we examine ATP release from PP-ATP and PP-ATP bilayers, demonstrating the electrodes can be used as ATP release devices. Cyclic voltammetry in solutions of both polycations and polyanions has been employed to illustrate ATP release from PP-ATP during redox switching and the inhibition of substantial spontaneous ATP release was further examined over an extended period of time. The dual ion transport concept of conducting polymer bilayers was combined with electrochemically stimulated ATP release, allowing modification of the surfaces of the films with heparin (HPN), a blood anticoagulant. ATP release from PP-ATP:PNMP-Cl bilayers was first examined using UV spectroscopy to compare the amount and rate of ATP release as a function of the thickness of each layer. PP-ATP:PNMP/HPN bilayers with varied PNMP/HPN thicknesses were prepared and characterized, demonstrating the control of ATP release with the possibility of biocompatibility. Supporting electrolyte concentration changes of the switching media were shown to be minimal using ion selective electrodes (Na^+ and Cl^-), yet confirmed the movement of anions or cations during redox switching.

Experimental Section

Pyrrole and *N*-methylpyrrole (Aldrich) were passed over neutral alumina until colorless before use. ATP disodium salt, heparin sodium salt (Sigma Chemical), poly(styrenesulfonate) sodium salt, and NaCl (Aldrich) were used as received. A poly(4-vinylpyridine hydrochloride) (PVPy·HCl) solution was prepared by dissolving an excess of PVPy (Reilly Chemical) in 0.1 M aqueous HCl.

PP-ATP inner films of varied thicknesses (150, 300, 750 nm) were prepared potentiostatically from 0.1 M pyrrole in 20 mM ATP aqueous solutions at +0.8 V (vs Ag/AgCl). These solutions have a pH of 3.4, where the ATP exists as tribasic ions. PNMP-Cl outer films of various thicknesses (150, 300, 450, 750 nm) were synthesized potentiostatically over the PP-ATP from 0.1 M NaCl and 0.1 M pyrrole aqueous solutions at +0.9 V (vs Ag/AgCl). PNMP/HPN outer films were prepared in a similar manner, using 0.1 M NMP and 10 mM HPN sodium salt aqueous solutions. The preparation of PNMP outer films was carried out as quickly as possible to avoid diffusion of the monomer into the bulk of PP-ATP inner film. The film thicknesses were calculated by the charge passed during electropolymerization.[8]

The working electrode of the electrochemical quartz crystal microbalance (EQCM)[9] used for chronocoulometric and microgravimetric studies was a gold-coated (0.71 cm^2) quartz crystal. In voltammetric, spectroscopic and ion-selective electrode (ISE) studies, a Pt button (0.02 cm^2) or Pt plate (0.50, 0.75 cm^2, or 6.25 cm^2) were used respectively as working electrodes. The counter electrode was a Pt plate with a Ag/AgCl reference electrode for gravimetric and voltammetric studies. Ag wire (+0.02 V vs Ag/AgCl in 0.1 M NaCl) was used as the quasireference electrode with a Pt plate as a counter electrode for spectroscopic and ISE measurements.

After thorough washing with distilled water, bilayers were placed in 0.1 M NaCl aqueous solutions and fully oxidized by applying +0.6 V (vs Ag wire) for 1 min. The potentials were then stepped to 0.0 V as an intermediate potential to reduce the outer film while maintaining the inner films oxidized. After 10 min at 0.0 V, the inner film was fully reduced by stepping the potential to −0.6 V to release ATP. ATP concentration changes in 3.5 mL of 0.1 M NaCl aqueous solutions vs. time were monitored in situ using a Cary 5E UV−vis−NIR spectrophotometer, while controlling electrode potentials with an EG&G Model 273 potentiostat/galvanostat.

For measurements of electrolyte (Na^+ and Cl^-) concentrations, bilayer films were placed in 5 mM NaCl aqueous solutions and equilibrated at 0.0 V (vs Ag wire) for 3 min. These films were thoroughly washed with 5 mM NaCl aqueous solutions and immediately immersed in 3 mL of 5 mM NaCl. The outer films were reoxidized at +0.6 V (vs Ag wire) for 10 min, and a portion of the solution was taken for the ISE measurements. The solution used for the ISE measurements was returned to the electrochemical cell and the potential was subsequently stepped to −0.6 V (vs Ag wire) to reduce the inner film. The electrolyte concentration was examined again for a portion of the solutions.

Results and Discussion

PP-ATP Single Films. In previous work,[3] we have described the electrochemically stimulated release of ATP from PP-ATP modified electrodes and compared this fast ion supply to relatively slow and limited spontaneous ATP exchange with external electrolytes at open circuit. Assuming no spontaneous ion exchange of PP-ATP films, Genies reported that PP-ATP exhibits an ion dependent electrochemical response during cycling in various electrolyte solutions.[10] PP-ATP films cycled in NaCl solutions showed two separate redox processes with increased electroactivity over those cycled in ATP solutions. This observation of two redox processes was attributed to sites accessible to only Cl^- ions, which are generated by ATP release. We have now separated these phenomena by cycling PP-ATP in cationic and anionic polyelectrolyte solutions and subsequently comparing these cyclic voltammograms to that cycled in a NaCl electrolyte solution.

Figure 1 shows cyclic voltammograms of PP-ATP (450 nm on a Pt button) cycled in 0.1 M (a) NaCl, (b) NaPSS, and (c) PVPy·HCl aqueous solutions. The more anodic redox process is suppressed when the film is cycled in NaPSS, while the more cathodic redox peaks disappear when cycled in PVPy·HCl. This indicates that the more anodic redox process is associated with anion-dominant transport and the more cathodic redox process with cation dominant transport. Since the PP-ATP would be expected to exhibit cation-dominant transport if the ATP

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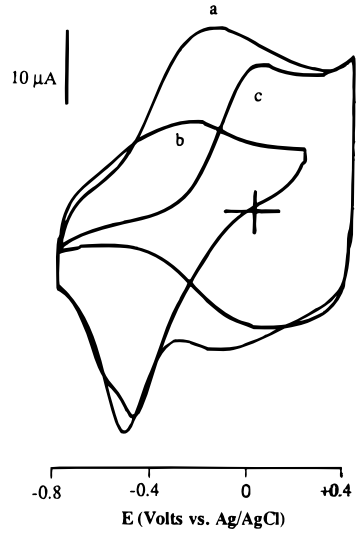


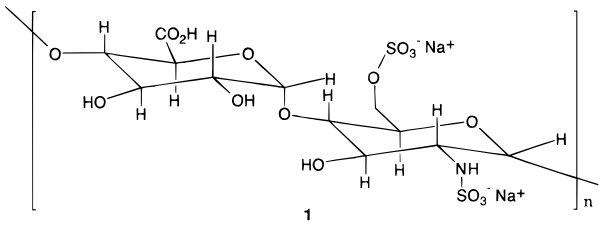
Figure 1. Cyclic voltammograms of PP-ATP (450 nm on a Pt button) in 0.1 M (a) NaCl, (b) NaPSS, and (c) PVPy·HCl (aq) solutions. $\nu = 100$ mV s^{-1}.

anions remained entrapped in the matrix, this suggests that ATP is released during electrochemical switching, causing the anion to become the dominant mobile species. It has been shown previously that multianionic species can be highly entrapped and immobile while some medium sized anions (e.g., tosylate) show mixed anion and cation transport.[11,12] In that ATP is a multiionic species (charge of -3 at pH $= 3-5$ and fully ionized above pH $= 7$)[13] and has a relatively high molecular weight (503 g mol^{-1} for ATP^{4-}), it is interesting to note that the anion is so easily ejected electrochemically. EQCM studies showed that, while the initial reduction is dominated by Na^+ penetration into the film, subsequent diffusion of ATP sodium salt out of the film occurs with ease.[3]

The inertness of PP-ATP to spontaneous ion exchange at open circuit was studied over an extended period of time. A PP-ATP film (1.5 μm thick) was placed in 3.5 mL of a 0.1 M NaCl aqueous solution, and UV absorbance increases of the electrolyte solution due to spontaneous ion exchange were monitored up to 12 days. Figure 2 compares the spontaneous release (curves b) with the UV absorbance increase due to electrochemically induced ATP release after cycling nine times between -1.0 V and 0.0 V (vs Ag wire) at 10 mV s^{-1} (curve a). Spontaneous ATP release is slightly activated after one day, but ultimately reaches a limiting value of ca. 10% of the electrochemically releasable ATP, as shown in the inset. This indicates that some loosely bound ATP can be spontaneously released. This is in contrast to a number of anionic dopants which can be spontaneously exchanged quantitatively.[14] We believe that the limited amount of spontaneous release is an important factor to consider in the construction of biological molecule release devices.

The maximum amount of electrochemically releasable ATP is a function of the amount of ATP contained in the film and not film thickness alone. This was confirmed by comparing results from two film thicknesses (0.5 and 1.5 μm) having the same material volume (i.e., constant number of electroactive sites). The ultimate UV absorbance in the electrolyte attributable to ATP release is equivalent, as is the fraction of electrochemically releasable ATP (ca. 80%). It should be noted that there will be a limiting thickness which yields 80% releasable ATP as very thick polypyrrole films are not completely electroactive.

PNMP/HPN Single Layer. Heparin (**1**) is a sulfonated polysaccharide which is generated by certain types of cells that are especially abundant in the lining of arterial blood vessels. It has a repeating unit of six



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sugar residues, each consisting of an alternating sequence of sulfate derivatives of *N*-acetyl-*d*-glucosamine and *d*-iduronate. Heparin is a very powerful inhibitor of blood clotting and aids in preventing the formation of clots in circulating blood.

We utilized this polyelectrolyte as a dopant during the electropolymerization of *N*-methylpyrrole. The use of polyelectrolytes as dopants for the electrosynthesis of PP and PNMP conducting composites yields films with an excess density of anionic sites on the film surface.[15] In the case of HPN, the film will present a surface to the switching medium that is potentially biocompatible. To confirm the fact that PNMP/HPN possesses cation dominant transport properties, mass changes of a PNMP/HPN film (300 nm) during redox cycles were monitored along with current responses. Figure 3 shows a reproducible mass loss during positive potential scans indicative of cation release. The cyclic voltammogram of PNMP/HPN shows an $E_{1/2}$ of +0.1 V (vs Ag/AgCl) which is significantly lower than that of PNMP with small mobile dopant anions ($E_{1/2} \cong +0.5$V). We believe this shift of $E_{1/2}$ results from the difference in ion-transport mechanism. The effect of the ion transport mechanism on $E_{1/2}$ has been reported for polypyrrole,[16] showing the separation of the two redox processes (more cathodic cation dominant (minor) and more anodic anion dominant (major) regions).

In Situ Spectroscopic Studies. PP-ATP:PNMP-Cl bilayers with varied thicknesses of inner and outer films were prepared on a Pt plate. After thorough washing with doubly distilled water, bilayer films were placed in a cell containing 3.5 mL of aqueous 0.1 M NaCl. The bilayers were equilibrated at +0.6 V for 1 min and subsequently stepped to 0.0 V to reduce the outer film while measuring the UV absorbance of the electrolyte near the film surface at the λ_{max} for ATP (260 nm) with time. UV absorbance changes during this

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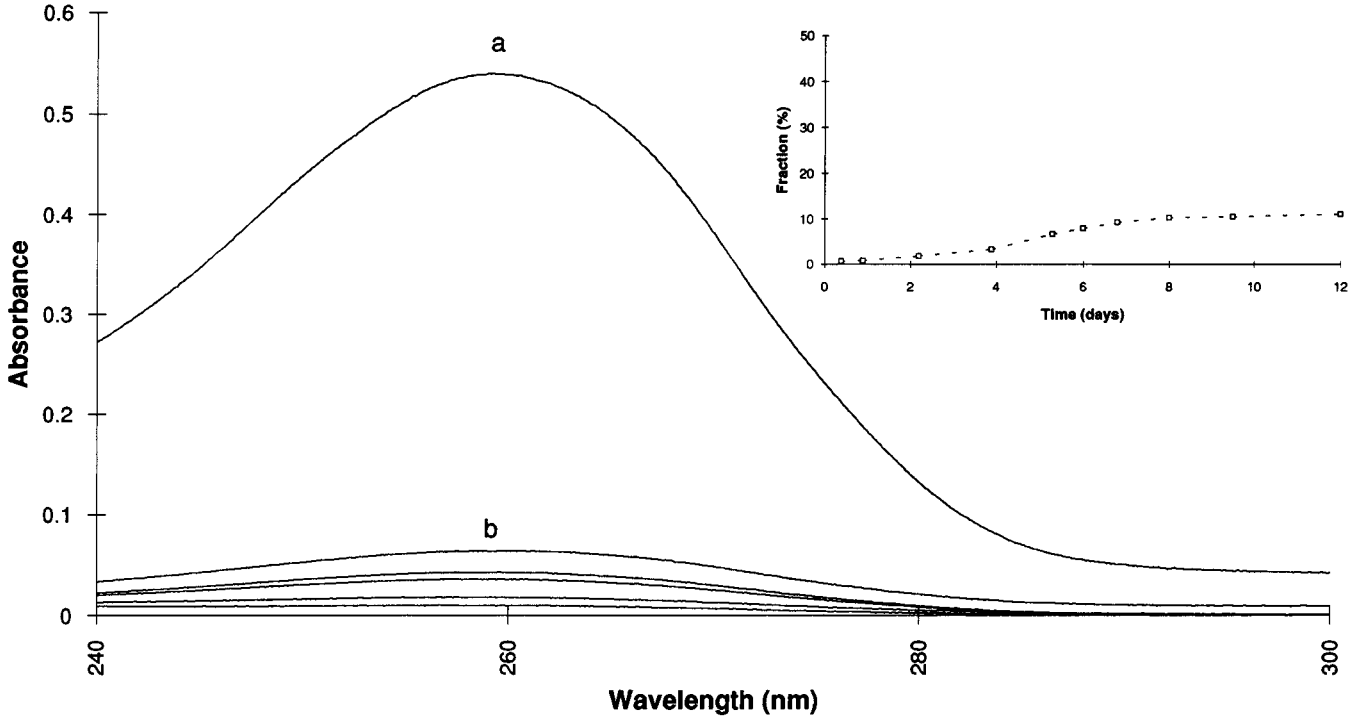

Figure 2. Comparison of UV absorbance increases between (a) electrochemically stimulated ATP release and (b) spontaneous ATP release. Curves under b shows the continuous increase of UV absorbances, measured after 2.2, 3.9, 5.3, 6.0, and 12 days. Inset shows fraction of spontaneously released ATP to electrochemically releasable ATP vs time.

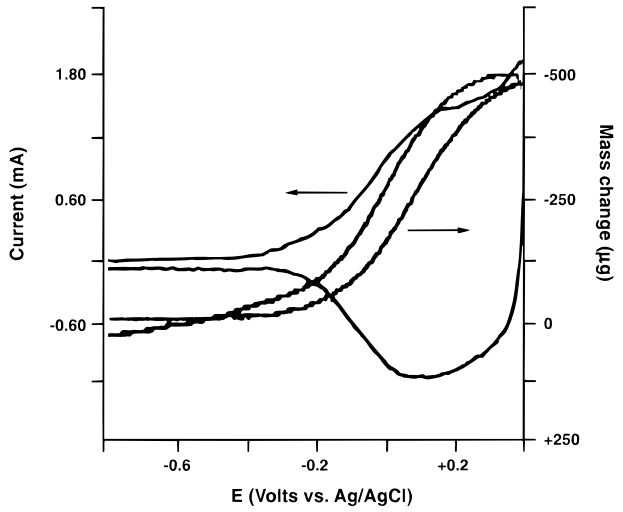

Figure 3. Voltammetric frequency and current responses of PNMP/HPN (300 nm on Au) in a 0.1 M NaCl (aq) solution. $\nu = 25$ mV s^{-1}.

process are negligible, as shown in Figures 4 and 5, indicating that the ATP remains entrapped in the film. During this reduction of the outer film there is Cl^- release from PNMP-Cl. This was confirmed as a mass loss using the EQCM. The mass decrease during reduction of a 300 nm thick PNMP-Cl outer film (ca. 0.34 μmol of total electroactive sites, assuming 30% doping level) was ca. 4.0 μg, indicative of 0.11 μmol of Cl^- released. This was further confirmed using a Cl^- ion selective electrode (ISE) during reduction of a film having the same thickness but larger area. A Cl^- concentration increase of 0.36 mM was measured when a PNMP-Cl outer film (ca. 3.0 μmol of total electroactive sites) was reduced in 3 mL of 5 mM NaCl. This corresponds to the release of 1.08 μmol of Cl^- from the film. Importantly, the EQCM and ISE results indicate

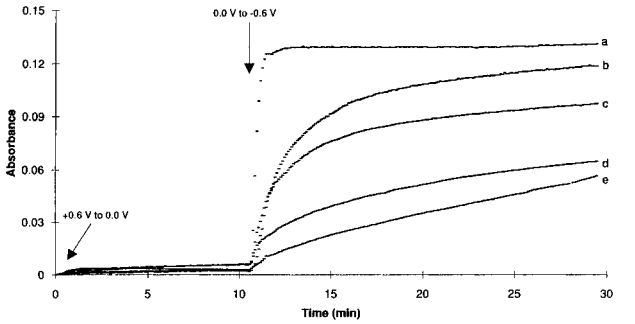

Figure 4. UV absorbance changes measured at 260 nm. PP-ATP (300 nm):PNMP-Cl [(a) no film, (b) 150 nm, (c) 300 nm, (d) 450 nm, (e) 750 nm] bilayers in 0.1 M NaCl subjected to potential steps as indicated.

that total supporting electrolyte concentration changes are negligible compared to the approximate 100 mM ionic strength of blood plasma. On the other hand, the small concentration change of anionic species can be important when highly bioactive anions (e.g., anionic drugs) are loaded into the outer film and released during an appropriate electrochemical stimulus.

We have reported the potential dependence of ATP release from PP-ATP single films and have shown that only small absorbance increases were observed at potentials ≥ -0.4 V.[3] The combination of this stability with bilayers similar to PP-ATP:PNMP-Cl will allow us to incorporate a variety of anions (or cations for PP-ATP:PNMP/PSS and PP-ATP:PNMP/HPN) into the outer films by potential cycling in an appropriate electrolyte. Those anions can be released at the first potential step followed by ATP release at the second potential step, yielding potential-dependent double ion release from a single electrode.

After the PP-ATP:PNMP-Cl bilayer electrode had equilibrated at 0.0 V, the potential was then stepped to

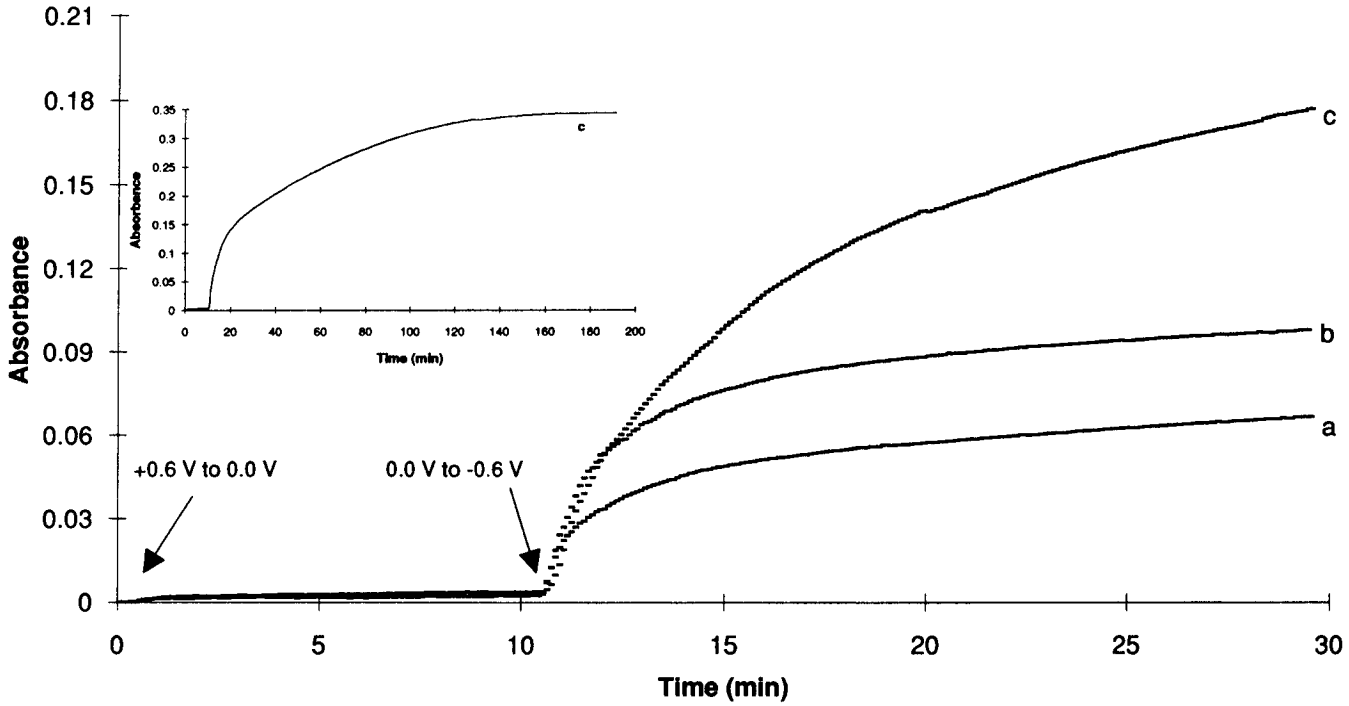

Figure 5. UV absorbance changes measured at 260 nm. PP-ATP [(a) 150 nm, (b) 300 nm, (c) 750 nm]:PNMP-Cl (300 nm) bilayers in 0.1 M NaCl subjected to potential steps as indicated.

−0.6 V to stimulate ATP release by reducing the inner film. Figure 4 shows immediate UV absorbance increases, with the extended time rate of release being a function of outer film thickness. It is evident that the ATP is released most rapidly when no outer film is present, quickly reaching a maximum within ca. 3 min (Figure 4a). Assuming a doping level of 0.27,[3] it can be inferred that PP-ATP inner films (300 nm thick), electropolymerized on a Pt plate (0.75 cm^2), are composed of 4.2×10^{-7} mol of repeat units and 3.8×10^{-8} mol of ATP. Using the extinction coefficient of 15.4×10^3 dm^3 mol^{-1} cm^{-1}, it can be concluded that 2.95×10^{-8} mol of ATP, or 78% of the initially incorporated ATP, was released, consistent with the value previously reported.[3] The time dependence of ATP release from PP-ATP:PNMP-Cl is inversely related to outer film thickness. For example, after 10 min at −0.6 V in Figure 4, 63 %, 51%, 30%, and 22% of the ATP is released for bilayers consisting of 150, 300, 450, and 750 nm thick PNMP-Cl outer films respectively. It should be noted that the ATP continues to slowly diffuse out of the film, suggesting relatively high ultimate release levels. The PNMP-Cl outer film serves to protect the inner film from direct contact with the electrolyte, while allowing the ATP to be transported into the electrolyte.

Figure 5 illustrates the UV absorbance changes in electrolytes containing bilayers consisting of varied PP-ATP inner film thickness overcoated with 300 nm of PNMP-Cl. A similar stepwise reduction experiment as described for Figure 4 was carried out. Again the electrodes are stable to ATP release with a potential step of +0.6 to 0.0 V. The initial ATP release rates are independent of PP-ATP film thickness. The ultimate amount of ATP released is proportional to the PP-ATP inner film thickness. The inset to Figure 5 shows that continuous and slow ATP release ultimately reaches the maximum value (80% of total ATP) regardless of the presence of the outer film.

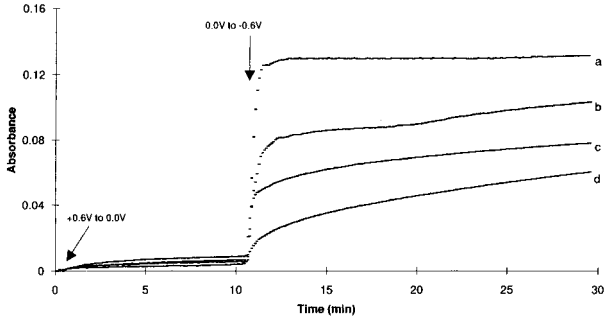

Figure 6. UV absorbance changes measured at 260 nm. PP-ATP (300 nm):PNMP/HPN [(a) no film, (b) 150 nm, (c) 300 nm, (d) 750 nm] bilayers in 0.1 M NaCl subjected to potential steps as indicated.

PP-ATP:PNMP/HPN bilayers were electrochemically prepared as a means of inducing cation-dominant transport with switching of the outer film, retaining ionic conductivity of the outer film in the neutral form as the polyelectrolyte salt remains entrapped, and to induce potential biocompatibility as PP/polyelectrolyte complexes are known to contain an excess density of ionic sites from the polyelectrolyte at the film:electrolyte interface. PP-ATP:PNMP/HPN bilayers with varied outer film thicknesses were subjected to the step potential reduction described for PP-ATP:PNMP-Cl and UV absorbance changes in the electrolyte measured with time. While negligible UV absorbance increase is seen during the outer film reduction, fast ATP release is observed upon reduction of the inner film, as shown in Figure 6. The amounts of released ATP at a certain time is inversely related to the outer film thickness, as the PNMP/HPN retards ATP transport. In comparison to PP-ATP:PNMP-Cl, the initial ATP release rate of these bilayers is markedly faster due to fast ion transport through the PP/HPN films. A significantly higher ionic conductivity of PP/PSS relative to poly(pyrrole perchlorate) has been reported using impedance spec-

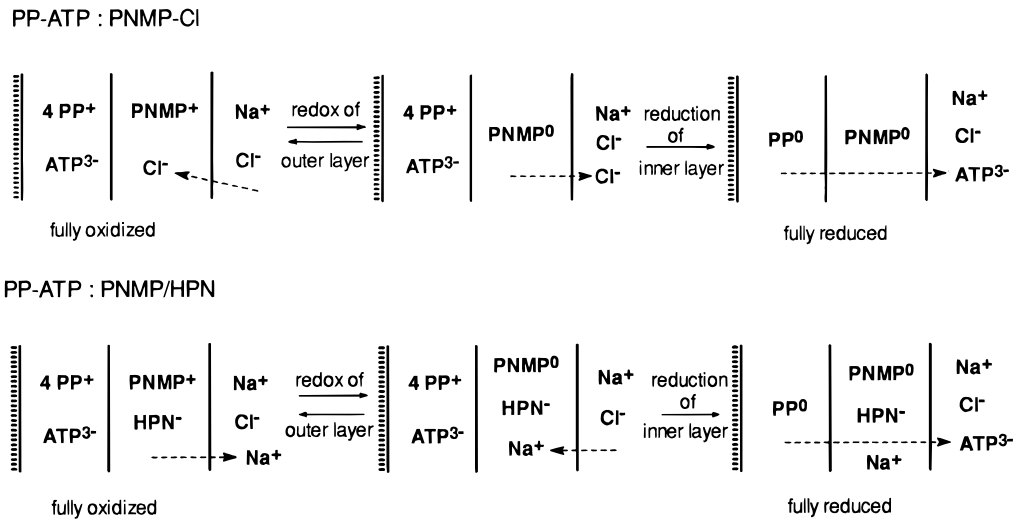

Figure 7. Schematic diagram showing the control of ion flux and electrochemically stimulated ATP release from PP-ATP bilayers.

troscopy and indicates that PP/polyelectrolyte composites to be the most useful as outer layers.[17]

Conclusions

The stability to spontaneous exchange processes and electrochemically stimulated release of ATP has been investigated for PP-ATP and PP-ATP bilayers. Spontaneous ATP release leveled off after 8 days, and ca. 10% of the electrochemically releasable ATP was released after 12 days, indicating that only surface bound ATP can be spontaneously exchanged with external electrolytes. Bilayers consisting of PP-ATP inner films and PNMP-Cl and PNMP/HPN outer films showed electrochemically stimulated ATP release proportional to PP-ATP inner film thicknesses and rates related to outer film thicknesses and compositions.

The bilayer concept is outlined in Figure 7. By constructing a bilayer of PP-ATP (or possibly other anionic biological molecules which can be electrochemically released) and PNMP-Cl (or possibly other anionic biological molecules as dopants), a single polymer-modified electrode can be used to absorb or emit bioactive anions by controlling the redox state of the films. The process can be carried out stepwise with the inner film acting as an extension of the electrode allowing the outer film's redox process to be accessed. By utilizing PP-ATP:PNMP/HPN bilayers, bioactive cations or electrolyte cations can be transported in and out of the outer film while presenting an HPN modified surface to the switching medium. This versatility will allow us to apply these bilayer electrodes through surface property control and to prepare polymer modified electrodes which emit useful anions or cations (in addition to ATP release) as a function of bilayer composition.

Acknowledgment. Financial support from the University of Florida, Division of Sponsored Research, and the National Science Foundation (CHE 9307732) is gratefully appreciated.

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