Studies on Electrochemical Oxidation Mechanisms of Polyamides Containing *N*-Methylpyrrole and *N*-Methylimidazole by Electrospray Ionization Tandem Mass Spectrometry

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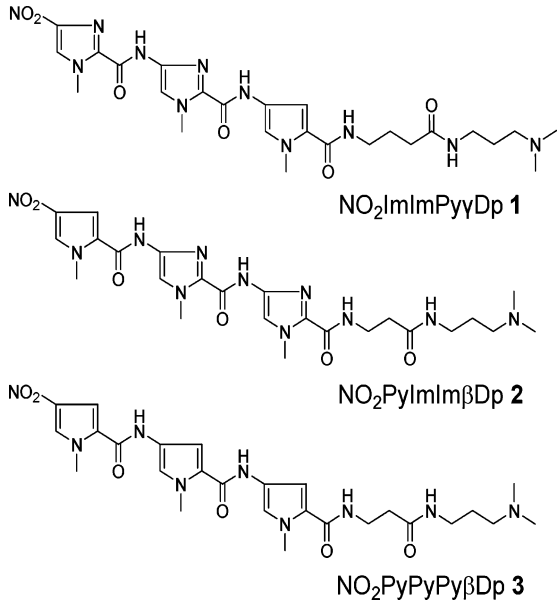
Electrochemical oxidization mechanism of polyamides containing *N*-methylpyrrole and *N*-methylimidazole was investigated by electrospray ionization tandem mass spectrometry (ESI-MS^n). The spectra of the oxidization products unambiguously revealed that one or two oxygen atoms are added to the polyamide after the bulk electrolysis. It was found that electrochemical oxidation preferably takes place on the imidazole ring of the polyamide with both imidazole and pyrrole to produce a carbonyl group on the rings. ESI tandem mass spectrometry has proven to be an excellent method for the structural identification of electrochemical oxidation products of DNA-recognizing polyamides.

Polyamides containing *N*-methylpyrrole and *N*-methylimidazole amino acids have received considerable attention for being cell-permeable and for the capability of specifically recognizing predetermined sequences of DNA and controlling gene expression.[1,2] Many techniques have been employed to trace and characterize this specific recognition, such as DNase titration,[3] NMR,[3] fluorescence,[4] and molecular dynamics simulations.[5] In general, all these techniques only provide information about the equilibrium or steady states of this recognition; however, to study the kinetics of polyamide−DNA interaction, real-time detection methods should be encouraged. As Bard reported, electrochemical method is an excellent real-time detection method to study the interaction of small molecules and DNA.[6,7] Here we investigated the electrochemical behavior of polyamides and proposed an electrochemical oxidization mechanism, which is essential for studying the interaction between polyamides and DNA by electrochemistry as well as developing biosensors further. In this paper, the electrochemistry of three polyamides (NO_2ImImPyγDp (**1**), NO_2PyImImβDp (**2**), and NO_2PyPyPyβDp (**3**) (Scheme 1, where Py = *N*-methylpyrrole, Im = *N*-methylimidazole, γ = γ-aminobutyric acid, β = β-alanine, Dp = *N*,*N*-dimethylaminopropylamine) synthesized[8] were investigated by differential pulse voltammetry first, and then the electrochemical oxidation mechanism is proposed by electrospray ionization (ESI) tandem mass spectrometry.

SCHEME 1: Structures of Polyamides 1, 2, and 3



Experimental Section

Differential Pulse Voltammetry (DPV). Differential pulse voltammetry (DPV) was performed on a CHI706 electrochemistry workstation (CHI Instrument Co., USA) using a one-compartment homemade electrolytic cell (volumetric capacity 10 mL) with a three-electrode system. A glassy carbon electrode with a diameter of 4 mm was used as the working electrode, a Pt coil was used as the counter electrode, and a saturated calomel electrode (SCE) served as the reference electrode. Prior to use, the glassy carbon electrode was polished with Micron Gamma Alumina (Buehler Co., USA, 0.05 μm) on a polishing chamois, resulting in a surface with a mirrorlike appearance. After mechanical cleaning, rinsing, and ultrasonic rinsing for 3 min in water, a cyclic voltammetry experiment was carried out in potassium ferricyanide solution to check the surface condition of the glassy carbon electrode; the peak separations (ΔE_p) must be about 70 mV. The differential pulse voltammetry conditions used were pulse amplitude 50 mV, pulse width 70 ms, and potential increasement 4 mV. All experimental data obtained

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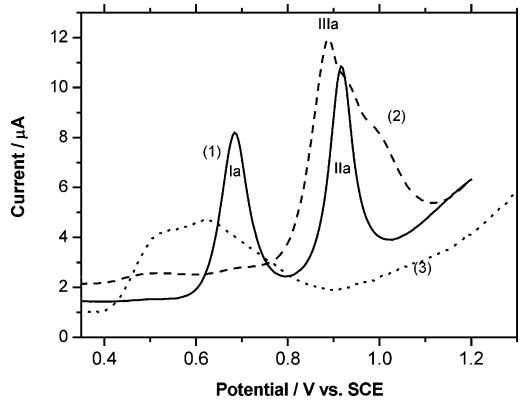
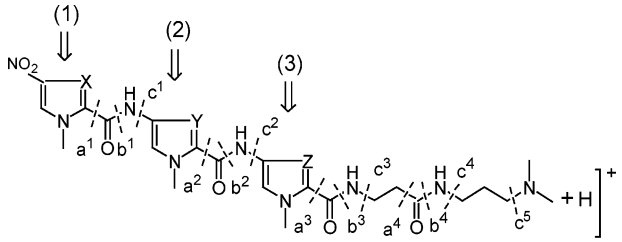


Figure 1. DPV of the polyamides at glassy carbon electrode with 5 $\times$ 10^{-5} mol/dm^3 concentrations in 0.2 M acetate buffer, pH 4.5: (1) NO_2ImImPyγDp (solid line); (2) NO_2PyImImβDp (dashed line); (3) NO_2PyPyPyβDp (dotted line).

SCHEME 2: Main Fragmentation Models of Polyamides



	X	Y	Z
1	N	N	C
2	C	N	N
3	C	C	C

came from the first scan of the DPV to avoid contamination on the electrode surface. Polyamide was dissolved in a pH 4.5, 0.2 M aqueous acetate buffer with 5 $\times$ 10^{-5} mol/dm^3 concentration. Twice-distilled water from a quartz distillation apparatus was used in all experiments. All experiments were performed at ambient temperature.

Bulk Electrolysis. Bulk electrolysis was performed on a potentiostat/galvanostat (Model 173, EG&G Co.) in a two-compartment cell with a three-electrode configuration. A Pt sheet served as the counter electrode introduced into one of the cell compartments, the other compartment involved a Ag/AgCl reference electrode, and another Pt sheet served as the working electrode. Prior to use, the working electrode, Pt sheet, was immersed in chromic acid and rinsed by water. The controlled-potential technique was employed in bulk electrolysis. Polyamide **1** was electrolyzed at 0.8 and 1.0 V for 24 h, respectively. Polyamides **2** and **3** were electrolyzed under 1 V condition for 48 h. After each electrolysis was complete, the product was analyzed by electrospray ionization (ESI) tandem mass spectrometry.

Mass Spectrometry. The ESI-MS spectra were recorded by a Finnigan LCQ mass spectrometer (Thermo Finnigan, San Jose, CA) for the identification of the electrolysis products. The capillary needle voltage was 4000 V and the source temperature was maintained at 200 °C; the solution was introduced into the electrospray source at a flow rate of 2 μL/min and ions were scanned with a scan speed of 5500 Da/s at unit resolution. Data acquisition and processing were achieved using the Finnigan Xialibur analysis system.

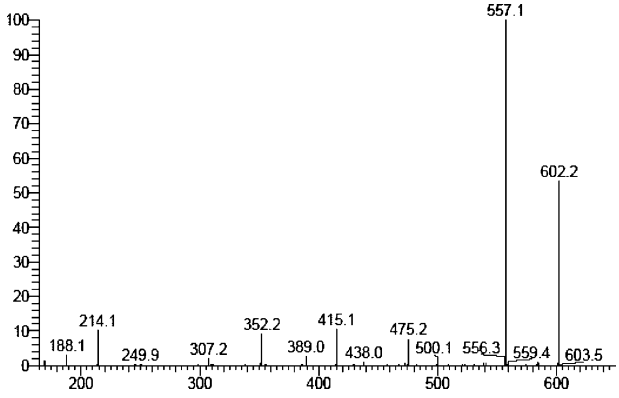


Figure 2. ESI-MS/MS spectrum of $[M + 16 + H]^+$ of polyamide **1** after electrolysis for 24 h at 0.8 V vs Ag/AgCl.

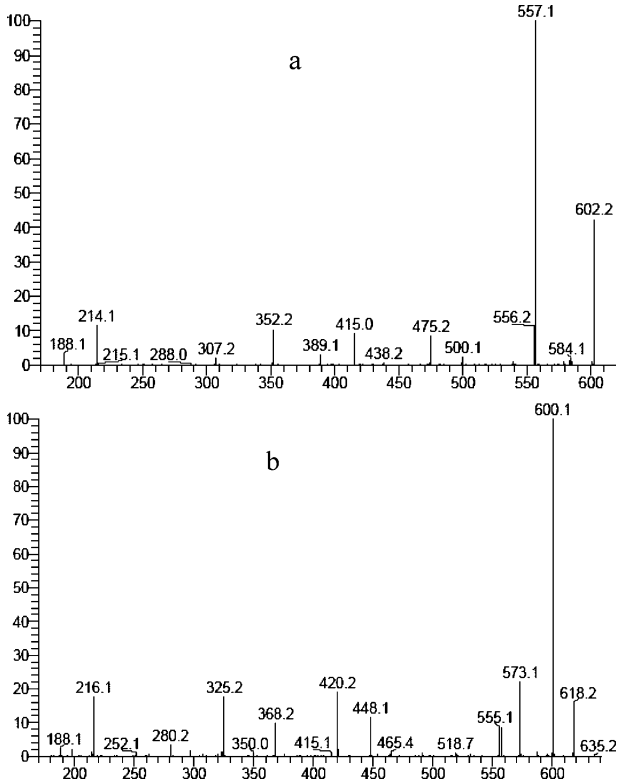
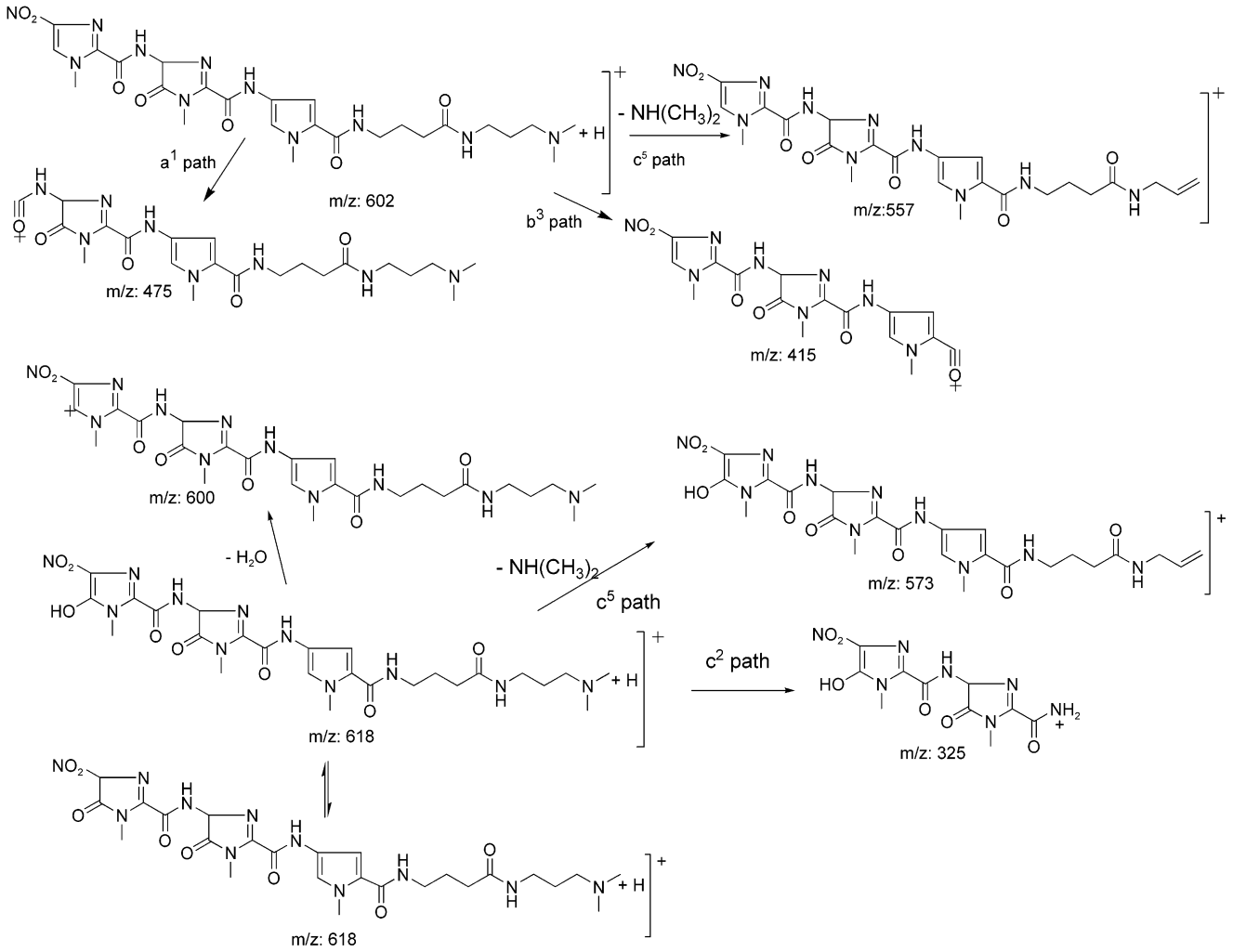


Figure 3. ESI-MS/MS spectra of $[M + 16 + H]^+$ and $[M + 32 + H]^+$ of polyamide **1** after electrolysis for 24 h at 1 V vs Ag/AgCl: (a) $[M + 16 + H]^+$; (b) $[M + 32 + H]^+$.

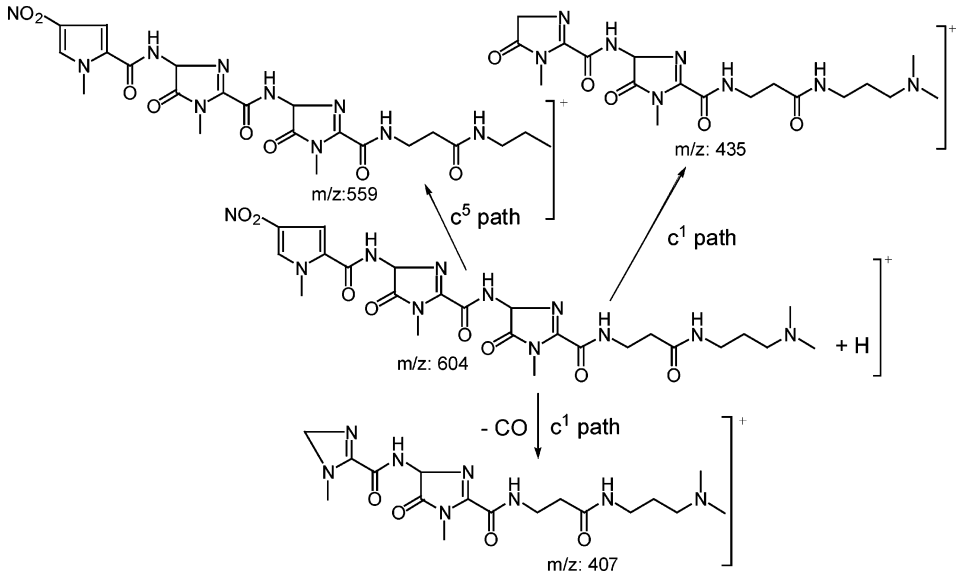
Results and Discussion

DPV of polyamides **1**, **2**, and **3** at the glassy carbon electrode are shown in Figure 1, which presents the electrochemical oxidation characteristic of the polyamides. In Figure 1, two anodic peaks are observed at 0.684 and 0.913 V for polyamide **1**; only one anodic peak with two shoulder peaks was obtained for polyamide **2** at 0.888 V; two small humps with a lower peak current can be seen at 0.52 and 0.62 V for polyamide **3**. These results indicate that the electrochemical activity of polyamide **3** is less than that of polyamides **1** and **2**. The numbers of peaks suggest that polyamide **1** was oxidized by two steps, while polyamide **2** may be oxidized by one step. Furthermore, the appearance of two shoulder peaks following IIIa indicates that the oxidation of the two imidazole rings have slight close peak potentials, and pyrrole ring in polyamide **2**

SCHEME 3: Main Fragmentation Pathways of $[M + 16 + H]^+$ ($m/z = 602$) and $[M + 32 + H]^+$ ($m/z = 618$) of Polyamide 1 after Electrolysis



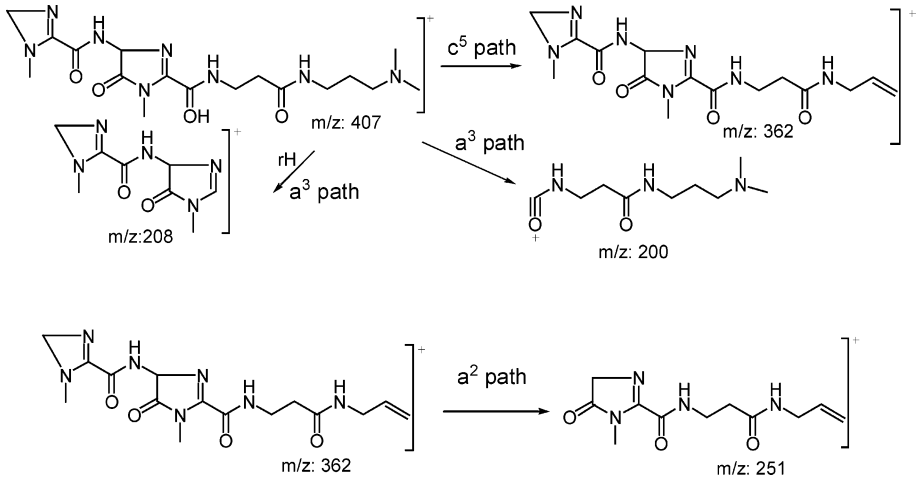
SCHEME 4: Main Fragmentation Pathways of $[M + 32 + H]^+$ at m/z 604 of Polyamide 2



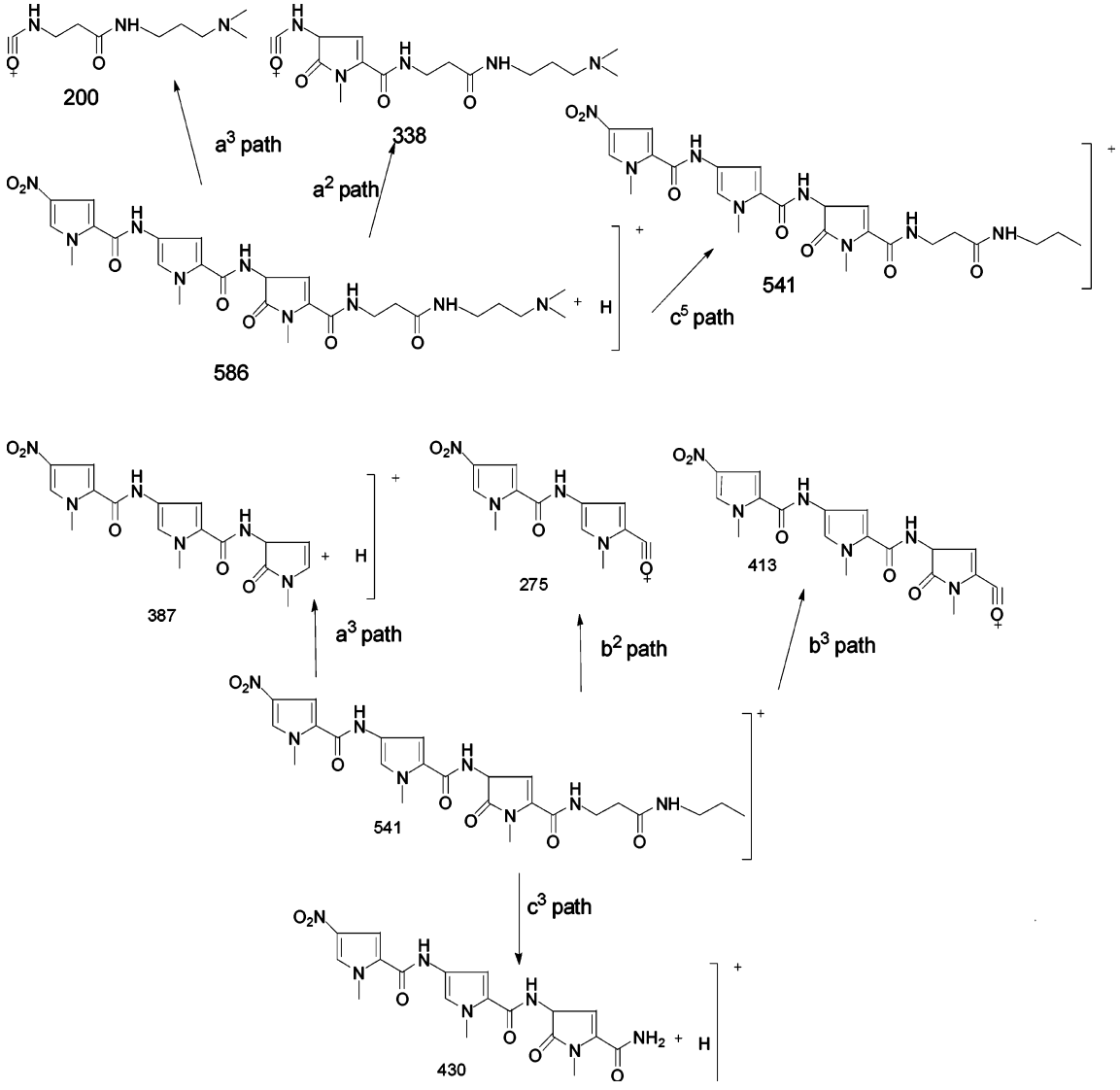
may also participate in the oxidation process at high potential. The peak area (IIIa) of polyamide **2** equals the sum of the two anodic peak areas (Ia and IIa) of polyamide **1** with the identical concentration 5×10^{-5} mol/dm^3, which suggests that the oxidation waves arise from the oxidation of the imidazole rings in polyamide. The Ia peak area is close to the IIa peak area in polyamide **1**; that means the two imidazole rings are oxidized one by one.

In previous work[9] we observed that the potentials of the three anodic peaks, Ia and IIa of polyamide **1** and IIIa of polyamide

SCHEME 5: Main Fragmentation Pathways of Ions at *m/z* 407 and 362



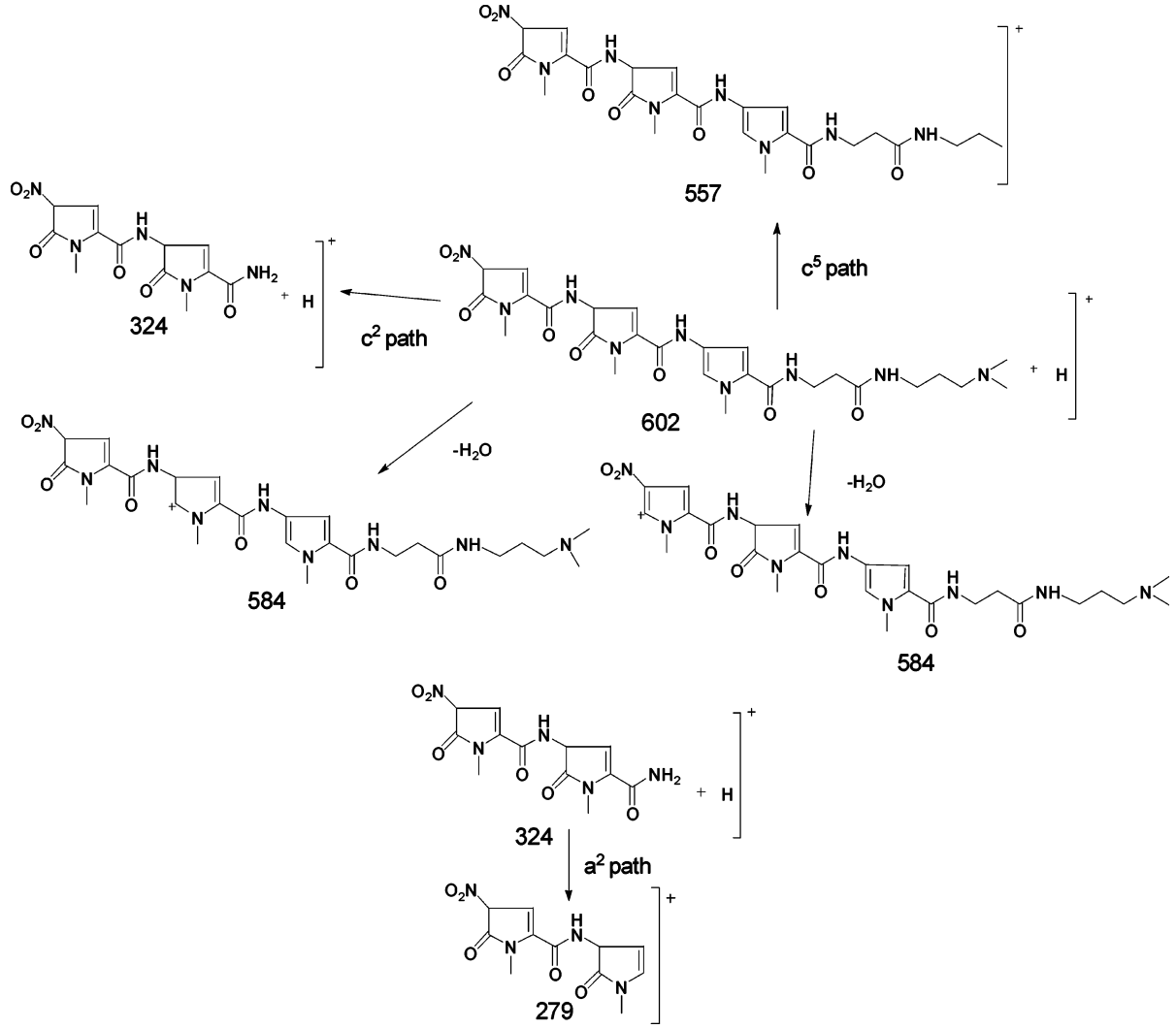
SCHEME 6: Main Fragmentation Pathways of $[M + 16 + H]^+$ at *m/z* 586 and Ion at *m/z* 541



2, depend on the solution pH, in which three potentials shift negatively with increasing pH. The three slopes of the fitted lines from $E-$pH curves are close to the theoretical value of -0.0591 V/pH unit, suggesting that the same number of electrons and protons is involved in the oxidation processes.

To further explore the mechanism of the electrochemical oxidation of the polyamides, electrospray ionization mass spectrometry (ESI-MS^n) was employed to analyze the oxidation products. The fragmentation modes of the mass spectra of polyamides **1**, **2**, and **3** can be rationalized by the a^n cleavage (the cleavage of the C$-$CO bond), the b^n cleavage (the cleavage

SCHEME 7: Main Fragmentation Pathways of $[M + 32 + H]^+$ at *m/z* 602 and Ion at *m/z* 324



of CO−NH bond), and the c^n cleavage (the cleavage of NH−C bond or C−N bond) ($n = 1-4$ or 5) in Scheme 2[10−12] (the number in parentheses refer to numbering of the ring).

After polyamide **1** was electrolyzed for 24 h at 0.8 V, the ESI-MS of the electrolysis product showed a significant ion at *m/z* 602 ($[M + 16 + H]^+$), which suggests that one oxygen atom was added to the polyamide molecule. Subsequently, under 1.0 V condition, the two ions at *m/z* 602 ($[M + 16 + H]^+$, abundance 100%) and 618 ($[M + 32 + H]^+$, abundance 43%) were observed in the ESI mass spectra, which means that in this 1.0 V electrolysis condition one oxygen atom and a second one were added to the polyamide, sequentially. Furthermore, tandem mass spectrometry (MS/MS) was used to study the structures of $[M + 16 + H]^+$ and $[M + 32 + H]^+$, the oxidation products of the polyamide. ESI-MS/MS spectra of the $[M + 16 + H]^+$ and the $[M + 32 + H]^+$ ions are shown in Figures 2 and 3. The fragmentation modes of the $[M + 16 + H]^+$ and the $[M + 32 + H]^+$ ions are described in Scheme 3.

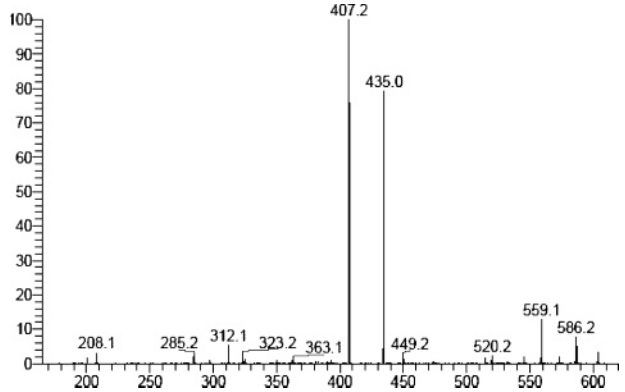
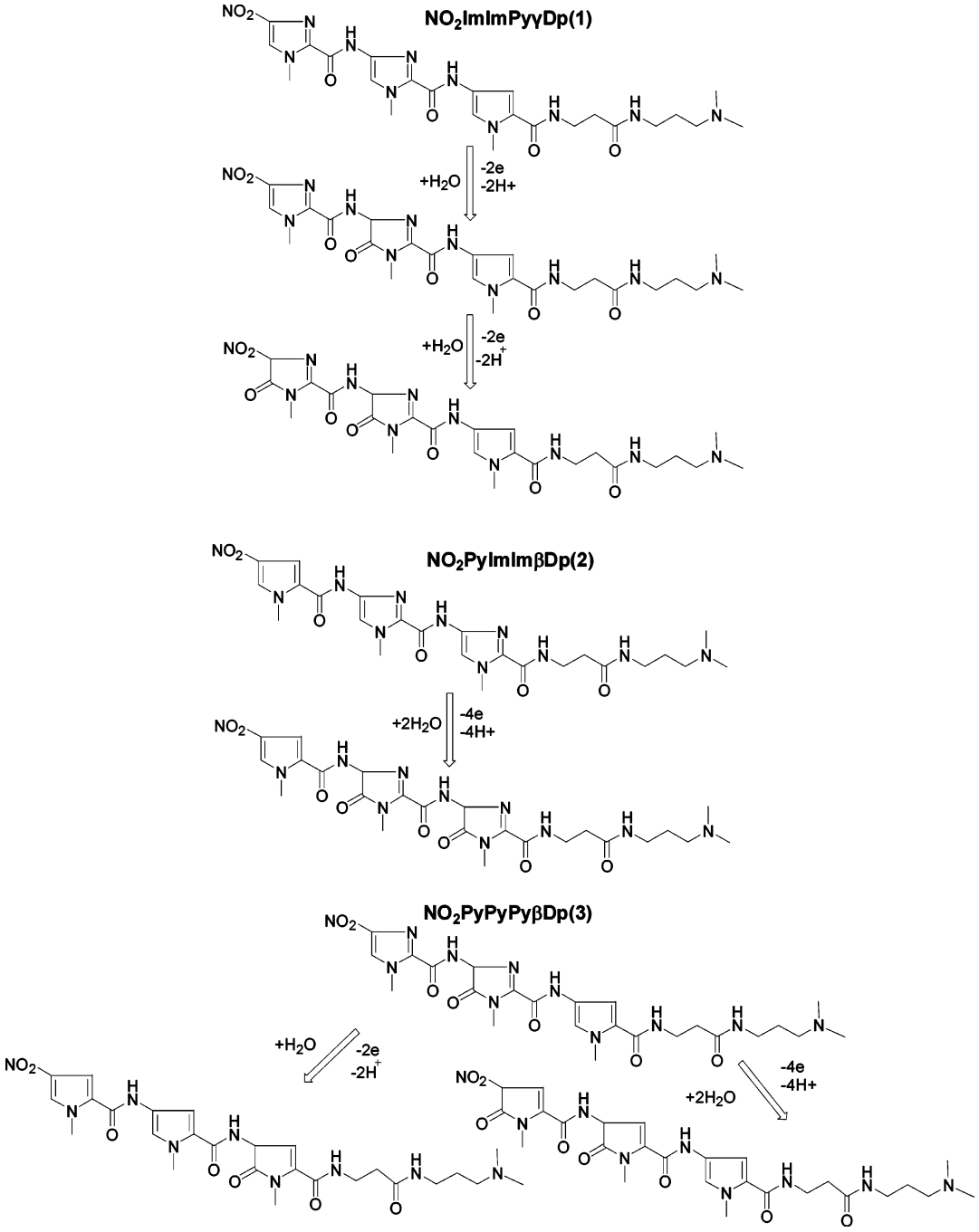


Figure 4. ESI-MS/MS spectrum of $[M + 32 + H]^+$ of polyamide **2** after electrolysis for 48 h at 1 V vs Ag/AgCl.

In the MS/MS spectrum of $[M + 16 + H]^+$, the ion at *m/z* 475 ($[M + 16 + H - 126]^+$) was produced from NO_2Im- extrusion by a^1 fragmentation, which means that the first oxygen atom was not added to the imidazole ring with a nitro group. In the MS/MS spectrum of $[M + 32 + H]^+$, the loss of PyγDp fragment from $[M + 32 + H]^+$ yields the ion at *m/z* 325 by c^2 cleavage (Figure 3b), which suggests the second oxygen atom is added into the outer imidazole (the one with a nitro group). Unlike $[M + 16 + H]^+$, a remarkable feature in MS/MS spectrum of $[M + 32 + H]^+$ is the fragment ion at *m/z* 600 by the loss of H_2O, which reveals that the carbonyl group on the outer imidazole ring would tautomerize to enol since there is an electron-withdrawing nitro group nearby.

Polyamide **2** was studied also by ESI-MS mass spectrometry after electrolysis to examine the electrochemical oxidation mechanism. The ESI-MS spectrum show one new significant ion at *m/z* 604 under 1 V condition for 48 h, which corresponds to $[M + 32 + H]^+$. That is, two oxygen atoms were added to

SCHEME 8: Electrochemical Oxidation Mechanisms of Polyamides at Glassy Carbon Electrode



polyamide **2** at the same time. According to the oxidation mechanism of polyamide **1**, we believe that the two oxygen atoms should be added into the two imidazole rings of polyamide **2**, respectively. An ESI-MS/MS spectrum of the ion at *m*/*z* 604 ([M + 32 + H]$^+$) is shown in Figure 4 for the examination of the structure of this ion. The main fragmentation pathways of the [M + 32 + H]$^+$ were analyzed (Scheme 4). The loss of NO_2PyCONH− fragment from *m*/*z* 604 ion yields a significant ion at *m*/*z* 435 (80%) by c^1 cleavage, which suggests that the two oxygen atoms were added into the 2 and 3 rings of imidazole, respectively.

To understand the fragmentation mechanism of oxidized polyamide **2** and the structure of [M + 32 + H]$^+$ explicitly, the ESI-MS^n spectra of the ions at *m*/*z* 407 and 362 were recorded (Figure 5). Scheme 5 depicts the main fragmentation pathways based on the spectra obtained. The decomposition of the ion at *m*/*z* 407 yields the ion at *m*/*z* 362 by c^5 cleavage. In the ESI-MS^4 spectrum of *m*/*z* 362, the main ion is observed at *m*/*z* 251 (100%), which corresponds to the a^2 cleavage by losing the four-atom ring (ring 2). The structure of the ion at *m*/*z* 251 proves this suggestion.

The ESI mass spectrum of polyamide **3** after electrolysis (1.0 V vs Ag/AgCl for 48 h) shows two new ions at *m*/*z* 586 ([M + 16 + H]$^+$) and 602 ([M + 32 + H]$^+$), which suggests that one or two oxygen atom(s) were added to the polyamide, respectively. The fragmentation pathways of the [M + 16 + H]$^+$ were analyzed based on ESI-MS/MS spectra (Scheme 6), which provided the structural information on the ion at *m*/*z* 586. In particular, the ion at *m*/*z* 338 corresponds to a^2 cleavage of [M + 16 + H]$^+$, and the ion at *m*/*z* 541 yields the ion at *m*/*z* 275 by the b^2 path. The appearance of the ions at *m*/*z* 338 and 275

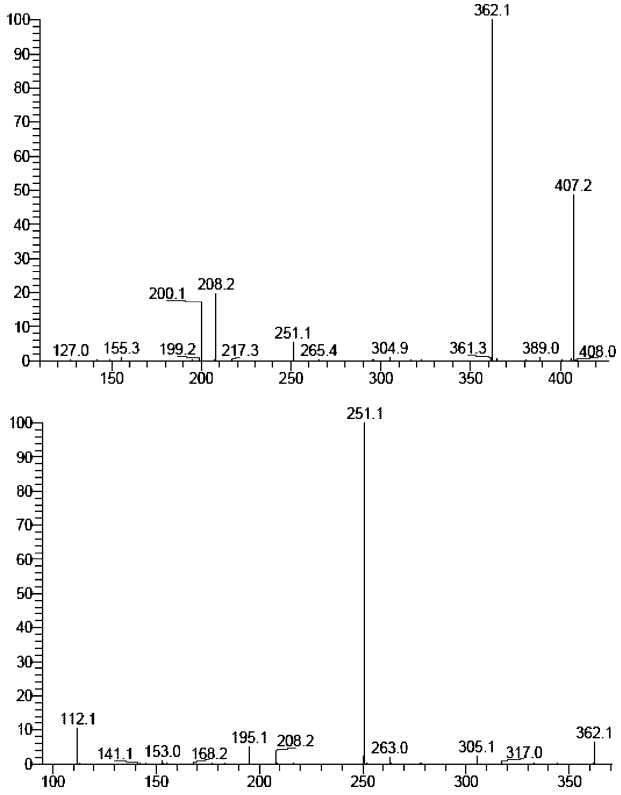


Figure 5. ESI-MS/MS spectra of ions at *m*/*z* 407 and 362.

indicates that one oxygen atom is added into the third ring of polyamide **3**.

Two oxygen atoms were added to polyamide **3** and formed another product to give the ion $[M + 32 + H]^+$. Scheme 7 shows main fragmentation pathways of $[M + 32 + H]^+$ at *m*/*z* 602 and the ion at *m*/*z* 324. The appearance of the ions at *m*/*z* 324 and at *m*/*z* 279 suggests that the two oxygen atoms were added to rings 1 and 2, respectively, of polyamide **3**.

ESI/MS^n spectra of $[M + 16 + H]^+$ and $[M + 32 + H]^+$ prove the position and character of the irreversible oxidation in polyamides **1**, **2**, and **3**. According to the ESI-MS^n study above, the electrochemical oxidation mechanism of the polyamides is described in Scheme 8. The double bond of imidazole or pyrrole loses two electrons to obtain one H_2O, followed by deprotonation, and finally forms carbonyl; the irreversible oxidation occurs preferably on the imidazole−pyrrole polyamide. It is clear that polyamide **1** is oxidized at 0. 8 and 1.0 V by two $2e^-/2H^+$ steps, while polyamide **2** is oxidized by a $4e^-/4H^+$ process at 1.0 V.

Conclusions

An electrochemical oxidation mechanism of polyamides is proposed by differential pulse voltammetry and ESI mass spectrometry. In this research, the ESI-MS/MS spectra of the oxidation products provide abundant evidence, which is very important for us to determine the detailed mechanism of electrochemical oxidation. The main fragmentation pathways of these ESI-MS^n spectra elucidate explicitly that one or two oxygen atoms are added to polyamide, and the double bond of imidazole ring is oxidized into a carboxyl group. Furthermore, the location of oxygen atom in the oxidized polyamides was identified by the fragmentation mechanisms of the polyamides. We now feel confident in using ESI tandem mass spectrometry to identify the structures of electrochemical oxidation products of DNA-recognizing polyamides.

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