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Electrogenerated Chemiluminescence Detection of Amino Acids Based on Precolumn Derivatization Coupled with Capillary Electrophoresis Separation

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A novel method for highly sensitive detection of primary and secondary amino acids with selective derivatization using acetaldehyde as a new derivatization reagent was proposed by capillary electrophoresis (CE) coupled with electrogenerated chemiluminescence (ECL) of tris(2,2′-bipyridine)ruthenium(II). The precolumn derivatization of these amino acids with acetaldehyde was performed in aqueous solution at room temperature for 1 h. Upon optimized derivatization, the ECL intensities and detection sensitivities of the amino acids were significantly enhanced by 20−70 times. Using four amino acids, arginine, proline, valine, and leucine, as model compounds, their derivatives could be completely separated by CE and sensitively detected by ECL within 22 min. The linear ranges were 0.5−100 μM for arginine and proline and 5−1000 μM for valine and leucine with the detection limits of 1×10^{-7} (0.5 fmol, arginine), 8×10^{-8} (0.4 fmol, proline), 1×10^{-6} (5 fmol, valine), and 1.6×10^{-6} M (8 fmol, leucine) at a signal-to-noise ratio of 3. The derivatization reactions and ECL process of amino acids were also proposed based on in situ Fourier transform infrared and ultraviolet spectrometric analyses.

Since capillary electrophoresis (CE) was presented in the 1980s,[1] it has been developed as an efficient separation technique due to its high efficiency, powerful resolution potential, relatively short analysis time, and small sample volume. Different kinds of detection methods compatible with CE, including UV−visible absorbance, refractive index, thermooptical absorbance, fluorescence, chemiluminescence, mass spectrometry, electrochemistry, and electrochemiluminescence or electrogenerated chemiluminescence (ECL), have been developed.[2] ECL detection, known as a useful analytical technique, has attracted considerable interest for development of highly sensitive and selective detection methods.[3] The most important ECL system is the reaction of ruthenium complexes, particularly tris(2,2′-bipyridine)ruthenium(II) ($Ru(bpy)_3^{2+}$) and its derivatives, which has become a powerful tool for the determination of different compounds containing tertiary amine or diketone groups,[4] amino acids,[5] oxalate,[6] various drugs,[7] and NADH.[8] Recently, the development of miniaturized ECL detection cells has extended greatly the application range of both ECL and CE.[3b,5,9] This work developed a novel, sensitive method for simultaneous detection of different amino acids by CE coupled with ECL of $Ru(bpy)_3^{2+}$.

Reactions between $Ru(bpy)_3^{3+}$ and tertiary amines have led to the development of ECL-based detection devices for a variety of biologically important molecules.[3b,10] According to the mechanism of $Ru(bpy)_3^{2+}$ ECL, $Ru(bpy)_3^{2+}$ is oxidized to form $Ru(bpy)_3^{3+}$, which further reacts with analyte, accompanied by light emission. Based on this mechanism, several subtle CE-ECL cells have been reported to detect amino acids,[5,11,12] peptides,[13] aliphatic amines,[3b,14]

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and polyamines.[15] Among these applications, most compounds determined are tertiary or secondary amines or their derivatives, because these compounds can give strong ECL signals in the reaction with $Ru(bpy)_3^{2+}$ without prior derivatization. The detection sensitivity of amines by utilizing their ECL reaction with $Ru(bpy)_3^{2+}$ increases in the order of tertiary > secondary > primary.[4,7d,16] The $Ru(bpy)_3^{2+}$-based ECL intensity of the compounds containing the primary amine groups is very low. Furthermore, the electrogenerated $Ru(bpy)_3^{3+}$ can react with water to produce background ECL,[17] which limits the detectability of amines, especially primary and secondary amines.[10b] Thus, few works on the $Ru(bpy)_3^{2+}$-based ECL determination of 20 standard amino acids comprising proteins have been reported except those for proline.[3b,9,11,12a] The determination of primary amines can usually be improved through the structural change of analytes or derivatization with different reagents to change primary amines to tertiary amines.[4b] This work proposed a new derivatization reagent for sensitive detection of primary amines by combining with a CE separation technique.

An ideal derivatization reagent should generally be stable, easy to obtain or synthesize, and can completely and quantitatively convert an analyte to a single and detectable derivative with minimal side reactions under mild reaction conditions in a short time. Furthermore, the reagent and derivative can be separated with a simple step.[4a] A variety of derivatization reagents such as divinyl sulfone,[4b] dansyl chloride,[18] 3-(diethylamino)propionic acid (DEAP),[4a] and naphthalene-2,3-dicarboxaldehyde[19] have been developed for $Ru(bpy)_3^{2+}$-based primary amine detection by HPLC- or CE-ECL.[20] However, excess derivatization reagent or its disintegrated compound often interferes with the detection. Although some derivatization reagents such as DEAP can be separated from their derivatives during the detection process,[4a] the presence of a strong ECL signal from the derivatization reagents is unfavorable to the separation and detection of multicomponent analytes. In the present study, the derivatives of amino acids with acetaldehyde were found to display strong ECL intensity in the presence of $Ru(bpy)_3^{2+}$ under mild conditions. Acetaldehyde could convert amino acids to corresponding derivatives within 1 h. It did not interfere with the determination of amino acids since no ECL signal of acetaldehyde was detectable. Using arginine, proline, valine, and leucine as model compounds of amino acids, their derivatives could be completely separated by CE with relatively high separation efficiency, and the proposed method showed much higher detection sensitivity than those reported previously.[4b,18a,b]

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EXPERIMENTAL SECTION

Reagents. All reagents and chemicals were commercially available and of analytical reagent grade. Tris(2,2′-bipyridyl)-ruthenium(II) chloride was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. β-Cyclodextrin (β-CD) (recrystallization before use), sodium sulfate, and amino acids were obtained from Shanghai Biochemical Co. All solutions were prepared with water purified in a Milli-Q System (Millipore, Bedford, MA). The stock solutions of amino acids were stored at 4 °C in a refrigerator. Acetaldehyde and formaldehyde (Shanghai Chemical Factory) solutions were adjusted to pH 8.0 with 0.1 M NaOH before use. The buffer solutions were prepared with sodium dihydrogen phosphate, disodium hydrogen phosphate, and sodium borate. All samples and buffer solutions were filtered through 0.22-μm cellulose acetate filters (Shanghai Xinya Purification Material Factory).

Apparatus. A programmable high-voltage power supply (0−20 kV, Remax Electronic Co., Ltd., Xi'an, China) was applied to perform the electrokinetic sample injection and electrophoretic separation. An uncoated fused-silica capillary with 50-cm length, 25-μm i.d.. and 360-μm o.d. was used for separation (Yongnian Optical Fiber Factory, Hebei, China). Before use, the capillary was flushed with 0.1 M sodium hydroxide solution overnight. The electrochemical measurements in CE-ECL experiments were carried out on a MPI-A multifunctional electrochemical analytical system (Xi'an Remex Electronic and Technological Co.) with a three-electrode system comprising platinum wire as the counter, Ag/AgCl (3.0 M NaCl) as the reference, and a 500-μm platinum disk as the working electrodes. The ECL emission was detected with a model BPCL ultraweak chemiluminescence analyzer (Institute of Biophysics, Beijing, China) in a pulse mode, which was sensitive to photons with a wavelength range of 200−800 nm. A CH Instruments model 600A analyzer (Austin, TX) with a 500-μm platinum disk working electrode was used to record the ECL curves.

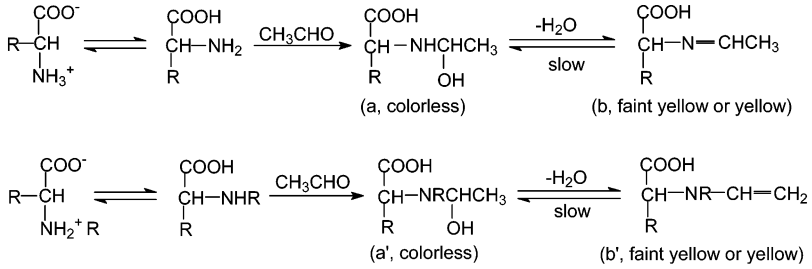
The ECL cell body was made of poly(methyl methacrylate) material, similar to those reported previously.[7b,d,9b] The gap between working electrode and capillary was controlled at 70 ± 5 μm.[9a,b] The lower layer of cell was made of a piece of optical glass through which the photons were captured by PMT, which was biased at 800 V. A 450-μL aliquot of 50 mM pH 8.0 phosphate buffer containing 5.0 mM $Ru(bpy)_3^{2+}$ was added to the cell for CE-ECL detection.

A Vector 22 FT-IR spectrophotometer (Bruker), equipped with a liquid nitrogen-cooled MCT detector and a vacuum system, and a UV2401 (Shimadzu) spectrophotometer were used for recording in situ Fourier transform infrared (FT-IR) and ultraviolet absorption (UV) spectra, respectively.

Precolumn Derivatization of Amino Acids. A 50-μL aliquot of standard amino acid or sample was mixed with 5 μL of acetaldehyde, and then 10 μL of 0.2 M PBS (pH 7.0) was added. The final concentration of acetaldehyde was 4.0 mM. The mixture was allowed to stand for 1 h at room temperature. The resulting mixture was subjected for analysis.

Procedure. Electrophoresis in the capillary was driven by a high-voltage power supply (12 kV, 4 μA), which was applied at the injection end with the detection cell held at ground potential through the separation capillary guide. In all experiments, the

Scheme 1. Derivatization Reactions of Amino Acids with Acetaldehyde



sample introduction was accomplished by electrokinetic injection for 10 s at 10 kV (10 kV × 10 s, ~4.5 nL).[9a] Before use, the capillary was flushed with purified water and the running buffer for 15 min by means of a syringe. The running buffer (pH 10.0) contained 25 mM phosphate, 25 mM borate, 6 mM β-CD, and 20 mM Na_2SO_4. After each run the capillary was regenerated by flushing with 0.1 M sodium hydroxide for 1 min followed by a 2-min water rinse and 2-min buffer rinse, and the electrode was treated with cyclic voltammetric scan in a potential range of −0.5 to 0 V at 100 mV s^{-1} for 2 min,[9b] to get better resolution and reproducibility.

The 0.4 mg of proline powder was mixed with ~20 mg KBr in an agate mortar and then pressed to a pellet for in situ FT-IR measurements of proline and its derivatives. The pellet was put into a glass tube with a CaF_2 optical window, which was connected to the vacuum system. The acetaldehyde vapor was introduced into the system for the derivatization reaction. During the reaction, the FT-IR spectra were recorded. The FT-IR spectrometer was modified to allow the infrared beam to transit the CaF_2 window. All spectral data were collected at a resolution of 2 cm^{-1} with an accumulation of 60 scans.

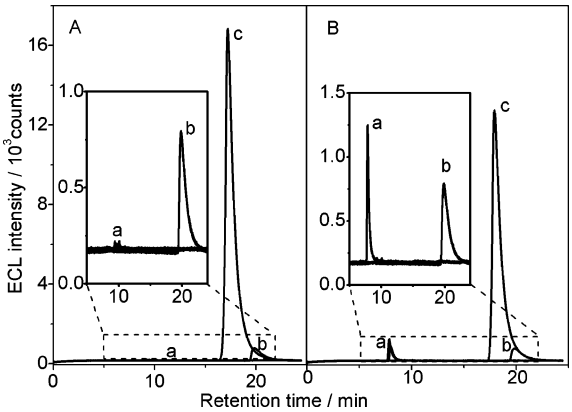


Figure 1. Electropherograms of (A) acetaldehyde (a), proline (b), and proline precolumn-derivatized with acetaldehyde at pH 7.0 (c); and (B) formaldehyde (a), proline (b), and proline precolumn-derivatized with formaldehyde at pH 7.0 (c). Concentration of proline, 100 μM; concentrations of both acetaldehyde and formaldehyde, 4.0 mM; running buffer, 25 mM phosphate + 25 mM borate + 4 mM β-CD + 20 mM Na_2SO_4 (pH 10.0); ECL cell, 50 mM pH 8.0 PBS + 5.0 mM $Ru(bpy)_3^{2+}$; injection, 10 kV for 10 s; separation voltage, 12 kV.

RESULTS AND DISCUSSION

Candidates for Derivatization. Both primary and secondary amines can react with different aldehydes to form secondary and tertiary amine derivatives, respectively, which can be oxidized by electrogenerated $Ru(bpy)_3^{3+}$ to produce stronger light emission.[4,18] The reaction mechanism of aldehydes with amino acids can be expressed as in Scheme 1.[21] The key step is nucleophilic addition to yield an amino alcohol intermediate, which then loses water slowly to give imine or enamine. As shown in Figure 1, after precolumn derivatization with both formaldehyde and acetaldehyde, the ECL intensity of proline in the presence of the electrogenerated $Ru(bpy)_3^{3+}$ increased greatly. No detectable ECL emission of acetaldehyde could be observed, while formaldehyde itself showed a weak ECL peak at the retention time of 7.8 min, which was far away from the peak of its proline derivative at 17.9 min. The derivatization of proline with acetaldehyde and formaldehyde resulted in the increase of ECL intensity by 26.4 and 19.6 times, respectively. Obviously, acetaldehyde as derivatization reagent favored the CE-ECL detection of standard amino acids.

ECL Curves for Cyclic Voltammetric Scan. The ECL curve of 5.0 mM $Ru(bpy)_3^{2+}$ solution (pH 8.0) during a cyclic voltammetric scan showed a very weak emission peak at ~+1.2 V (curve a in Figure 2), which resulted from the ECL reaction of $Ru(bpy)_3^{2+}$ with water.[17] Upon addition of 2.0 mM acetaldehyde, the emission peak increased twice, which was undetectable in CE-ECL experiments due to the presence of noise (as shown in Figure 1). Upon addition of 10 μM proline, 50 μM arginine, and 50 μM leucine to the $Ru(bpy)_3^{2+}$ solution, the ECL emission increased from 0.86 au to 27.6, 3.38, and 2.63 au, respectively (curve b in Figure 2). After derivatization of these amino acids with acetaldehyde, the right emission increased significantly. The ECL intensity for the derivatives of 2.0 μM proline, 10 μM arginine, and 50 μM leucine was 200.2, 47.6, and 49.2 au, respectively (curve c in Figure 2), which were much larger than those of corresponding amino acids before derivatization. The derivatization of amino acids with acetaldehyde could increase the ECL detection sensitivity of amino acids by 20−70 times. Since the applied detection potential must be larger than the oxidation potential (1.2 V) of $Ru(bpy)_3^{2+}$ for the production of ECL,[22] it was concluded from Figure 2 that the detection potential should be fixed at +1.25 V, at which the ECL intensity is the stongest.

Derivatization Reaction and Mechanism of ECL. The reaction time of amino acids with acetaldehyde affected the ECL intensity greatly. Upon addition of acetaldehyde to proline, the ECL signal increased quickly in the initial 30 min (Figure 3A), and the ECL intensity reached a maximum value at the reaction time of 1 h. After 2 h, the ECL signal decreased slowly and the

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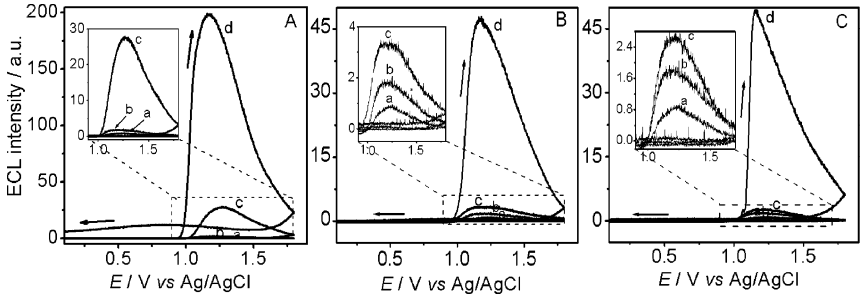


Figure 2. ECL curves for cyclic voltammetric scan at 100 mV s^{-1}. Curve a: 50 mM pH 8.0 PBS containing 5.0 mM $Ru(bpy)_3^{2+}$. Curve b: (a) + 2.0 mM acetaldehyde. (A) (a) + 10 μM proline (c) and (a) + 2.0 μM proline after derivatization (d). (B) (a) + 50 μM arginine (c) and (a) + 10 μM arginine after derivatization (d). (C) (a) + 50 μM leucine (c) and (a) + 50 μM leucine after derivatization (d).

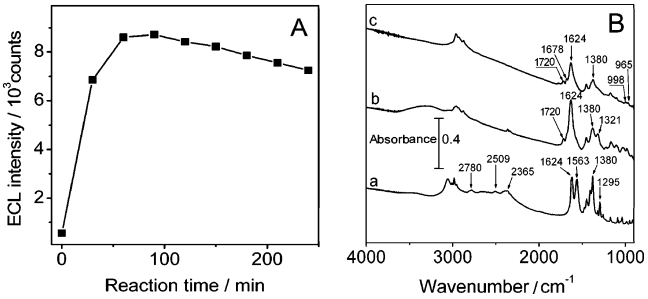


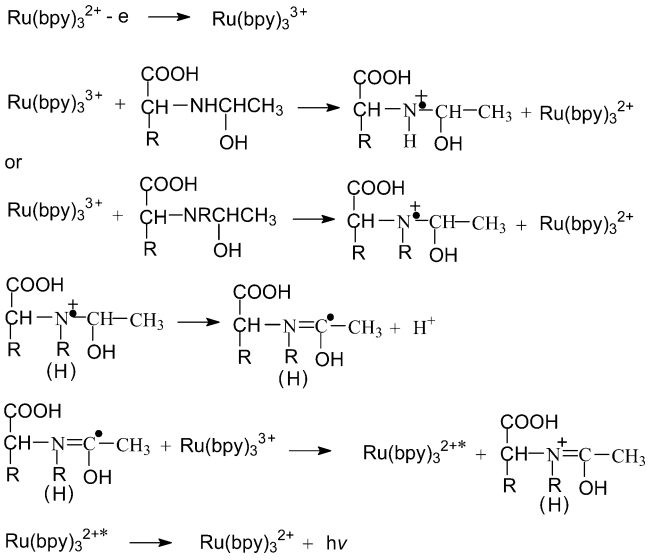
Figure 3. Effect of reaction time of 50 μM proline with 2.0 mM acetaldehyde on ECL intensity (A) and in situ FT-IR spectra (B) of proline mixed in KBr pellet in a vacuum (a), the proline mixed pellet after introducing acetaldehyde vapor for 1 h (b), and the product of b after heating for 1 h at 80 °C in a vacuum (c).

solution color became yellow gradually with the increasing reaction time. The maximum ECL intensity could retain for ~1 h, which was sufficient for CE-ECL detection. Thus, the precolumn derivatization time of 1 h was chosen for detection of amino acids.

The relation between the ECL intensity and reaction time coupled to the change in solution color suggested the formation of an amino alcohol intermediate in the reaction period of 1 h and final yellow imine or enamine product with a very slow reaction in solution. In weak acidic and neutral medium, the intermediate was relatively stable. The formation of an amino alcohol intermediate and imine or enamine product could be demonstrated from the in situ FT-IR spectra at different times.

In situ FT-IR investigation can permit direct examination of the intermediate reaction steps. As shown in Figure 3B, proline under vacuum condition showed observable absorption peaks corresponding to the stretch mode of COO^- at 1624 cm^{-1}, the bend mode of NH at 1563 cm^{-1} (δ_{N-H}), 2780, 2509, and 2365 cm^{-1} (ν_{N-H}), and the stretch mode of C−N at 1295 cm^{-1} (ν_{C-N} with NH) (curve a). The peak at 1380 cm^{-1} came from the bend mode of $-CH_2-$ in proline molecule. After introduction of acetaldehyde vapor for 1 h at room temperature, those peaks at 1563 cm^{-1}(δ_{N-H}), 2780, 2509, and 2365 cm^{-1} (ν_{N-H}) disappeared, and a new peak appeared at 1321 cm^{-1} (curve b), which corresponded to the stretching mode of C−N (ν_{C-N} without NH), indicating that the C−NH was converted to C−N and the amino alcohol intermediate was formed. The new peak occurred at 1720 cm^{-1} attributed to the adsorption of acetaldehyde on the sample pellet. To accelerate the followed reaction, the temperature of this system was increased to 80 °C. After this system was held at 80 °C for another hour, several new peaks appeared around 1678, 998, and 965 cm^{-1} (curve c), which were characteristic of the stretch mode of C=C and bend mode of $=CH_2$, respectively, suggesting that a new compound, enamine, was formed with loss of one H_2O molecule.

Scheme 2. ECL Process for Precolumn Derivatization Detection of Amino Acids with $Ru(bpy)_3^{2+}$



According to the ECL mechanism of the $Ru(bpy)_3^{3+}$/amine system,[3a,15,17] the formation of a final yellow imine or enamine product was unfavorable to the ECL detection of amino acids due to the low possibility to form $-N^+R=C=CH_2$ or $-N=C=CH_2$ in the present conditions, while the amino alcohol intermediate or the secondary or tertiary amine derivative could be oxidized by electrogenerated $Ru(bpy)_3^{3+}$ to form $-N^+R=COH-CH_3$ or $-N=COH-CH_3$ and produce the excite state of $Ru(bpy)_3^{2+}$, $Ru(bpy)_3^{2+*}$. Thus, the ECL process could be expressed as Scheme 2.

Optimization of Derivatization Conditions for Amino Acids. Proline was used as a representative amino acid to optimize the derivatization conditions. The pH value of the solution affected the ECL intensity of proline with a maximum intensity at pH 7.0 (Figure 4A). The pH dependence of ECL intensity was attributed to the formation of an amino alcohol intermediate. Low pH condition was unfavorable to the initial nucleophilic attack on the aldehyde by the lone-pair electrons of the amine group, which could not occur when too much acid was present.[21] In a weak acidic medium, amine was a more reactive nucleophile than that of complete protonation at low pH; thus, the ECL intensity increased with increasing pH value up to 7.0, which is near the

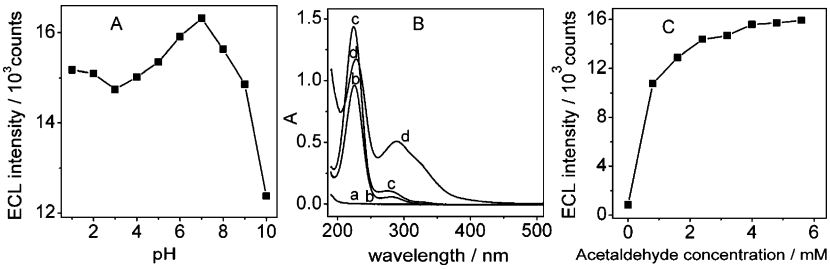


Figure 4. Effects of pH (A) and acetaldehyde concentration (C) for precolumn derivatization of 100 μM proline on ECL intensity and UV absorption spectra (B) of 50 μM proline (a), 2.0 mM acetaldehyde (b), and 50 μM proline after adding 2.0 mM acetaldehyde for 1.0 h at pH 7.0 (c) or pH 9.5 (d). Other conditions are the same as in Figure 2.

isoelectric point of proline. As shown in Scheme 1, addition to the aldehyde carbonyl group by the neutral amine nucleophile gave a dipolar tetrahedral intermediate, followed by proton transfer from nitrogen to oxygen, yielding a nonpolar amino alcohol (product a or a′). As mentioned above, the dehydration of the amino alcohol intermediate then gave imine or enamine (product b or b′) gradually, which decreased the ECL signal. The reaction rates of both the nucleophilic attack and the dehydration increased with the increasing pH value. At higher pH, the solution color also changed in shorter time and the ECL signal decreased when the same reaction time was used.

Figure 4B shows the UV spectra of several solutions. In the studied pH range, proline does not show any observable UV absorption (curve a), while 2.0 mM acetaldehyde displays a peak with the absorbance of 0.851 at 225 nm and a weak absorption around 283 nm with the maximum absorbance of 0.032 (curve b). These absorbances correspond to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the carbonyl group of acetaldehyde, respectively. Upon addition of 50 μM proline to 2.0 mM acetaldehyde, the absorption peak at 225 nm increased, which was attributed to the overlap of the absorption peaks for $\pi \rightarrow \pi^*$ transition of acetaldehyde and n $\rightarrow \sigma^*$ transition of the intermediate, amino alcohol (product a or a′).[23a] At pH 7.0, the maximum absorbance at 225 nm increased from 0.851 to 1.44, and the weak absorption around 283 nm increased from 0.032 to 0.112 after adding proline for 1 h (curve c). At pH 9.0, the mixture of 50 μM proline and 2.0 mM acetaldehyde showed a UV spectrum different from that at pH 7.0. After being mixed for the same time (1 h), the absorbance peak at 225 nm shifted slightly to 228 nm and the maximum absorbance was only 1.18 due to the decrease of amino alcohol concentration (curve d); the weak absorption around 283 nm was 0.521, much larger than that at pH 7.0. Obviously, the new weak absorption came from the $\pi \rightarrow \pi^*$ transition of the final product (product b or b′).[23b,c] These results indicated that the final product of imine or enamine was produced faster in alkaline medium, leading to a weaker ECL signal. To obtain the sensitive and stable ECL signal, as a compromise, pH 7.0 was selected as an optimal derivatization condition.

Effect of acetaldehyde concentration on the reaction was examined in the range of 0−6 mM (Figure 4C). With the increasing acetaldehyde concentration, the ECL intensity increased quickly and trended to a maximum value when the concentration was more than 4.0 mM, which was selected as the optimal concentration of acetaldehyde for precolumn derivatization of amino acids.

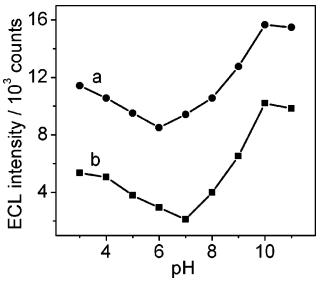


Figure 5. Effects of pH of running buffer on ECL intensity of 100 μM proline (a) and 100 μM arginine (b) after precolumn derivatization. Other conditions are the same as in Figure 2.

pH Values of Running Buffer and ECL Detection. For the $Ru(bpy)_3^{3+}$/amine system, the pH of detection solution mainly affects the potential window and hence the efficiency of Ru^{2+} electrooxidation. With the increasing pH, the ECL intensity for these analytes increased and showed the maximum intensity in the pH range of pH 7.5−9.5, which was in agreement with the usually optimum pH value of 8−9.[7b,24] This work used pH 8.0 for ECL detection and examining the pH dependence of the ECL intensity on the running buffer.

With the increasing pH of running buffer from 7.0 to 11.0, the ECL intensity of both proline and arginine derivatives increased and then decreased, the maximum ECL intensity occurred at pH 10.0 (Figure 5). Although the ECL intensity increased with decreasing pH when pH was less than 6.0, the separation become worse due to the adsorption of amino alcohol on the fused-silica capillary wall. Moreover, the CE-ECL system showed the strongest signal at pH 10.0; hence, this value was selected as the pH of running buffer. The running buffer did not change the pH of the detection buffer because only a small amount of running buffer flowed into the ECL cell.[7b]

The preliminary test indicated that borate buffer could lower the background signal and shorten the retention time, and PBS could give a high ECL signal; thus, this work chose 25 mM PBS and 25 mM borate solution as the running buffer. Besides that,

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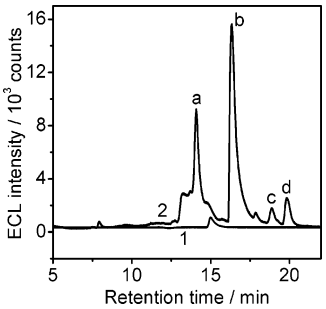

Figure 6. Electropherograms of standard samples containing 100 μM arginine (a), 100 μM proline (b), 100 μM valine (c), and 100 μM leucine (d) after (1) and before (2) precolumn derivatization. Other conditions are the same as in Figure 2.

20 mM Na_2SO_4[17,25,26] and 6 mM β-cyclodextrin[27−30] were added in the running buffer to restrain the adsorption of samples and improve the separation.

Calibration Curves and CE-ECL Detection of Amino Acids. Figure 6 shows a typical electropherogram of the mixture of four amino acids (arginine, proline, valine, leucine). The electropherogram of these amino acids without derivatization showed only one peak (curve 1) corresponding to proline. After precolumn derivatization, the mixture could be separated well, and four peaks corresponding to the four amino acids respectively occurred on the electropherogram (curve 2). The retention time of their derivatives was within 22 min. The other peaks (the prepeaks on a and the postpeak on b) were contributed to a small quantity of final products of the derivatization reaction, which could also react with $Ru(bpy)_3^{2+}$ to give the ECL response. The ECL intensity of the four amino acids increased linearly with the increasing concentrations of amino acids. The linear ranges were from 5×10^{-7} to 1×10^{-4} M for arginine ($R = 0.9995$, $n = 6$), 5×10^{-7} to 1×10^{-4} M ($R = 0.9998$, $n = 6$) for proline, 5×10^{-6} to 1×10^{-3} M ($R = 0.9993$, $n = 6$) for valine, and 5×10^{-6} to 1×10^{-3} M ($R = 0.9984$, $n = 6$) for leucine. The detection limits were 1×10^{-7} (0.5 fmol), 8×10^{-8} (0.4 fmol), 1×10^{-6} (5 fmol), and 1.6×10^{-6} M (8 fmol) for arginine, proline, valine, and leucine, respectively. The concentration of $Ru(bpy)_3^{2+}$ in the range of 1.0−10 mM did not influence the detection limits. When the concentration of $Ru(bpy)_3^{2+}$ was from 10 to 25 mM the noise of the ECL baseline increased, which decreased the sensitivity. The detection limits were lower than those reported with CE-ECL system, such as 1.2, 50, and 25 μM for proline, valine, and phenylalanine,[5b] 0.2[5a] and 0.16[9a] μM for proline with the same concentration of $Ru(bpy)_3^{2+}$, respectively, 1[12b] and 0.9 μM[13] for proline at 1.0, and 20 mM $Ru(bpy)_3^{2+}$, respectively, and much lower than those of 2 pmol for amino acid derivatives by dansyl chloride,[18b] DEAP,[4a] and DVS,[4b] 70 fmol for histamine, and 1−30 pmol for primary amines.

To demonstrate the suitability of the proposed method to analyses of real samples, the precision study were performed utilizing composite injection samples of five amino acids (arginine, histidine, proline, valine, leucine). The typical electropherogram of the sample was obtained after the precolumn derivatization procedure. Under optimal conditions, the separation of these five amino acid derivatives was satisfactorily achieved. The relative standard deviations of the peak heights of five amino acids with nine replicative injections were less than 4.8%. The recoveries of five amino acids in injection samples were between 92 and 101%.

CONCLUSIONS

This work proposes a novel derivatization reagent for highly sensitive ECL detection of primary and secondary amino acids coupled with capillary electrophoresis separation. Using a classical $Ru(bpy)_3^{3+}$/amine system and a simple precolumn derivatization by acetaldehyde under gentle conditions, the ECL intensity and detection sensitivity of the amino acids can be increased by 20−70 times. The ECL emission is produced from the reaction between electrogenerated $Ru(bpy)_3^{3+}$ and the amino alcohol intermediate of the derivatization reaction, which is formed at pH 7.0 in a derivatization period of 1 h. The separation of the analytes from excess derivatization reagent itself is unnecessary due to the undetectable signal of the reagent. Upon the precolumn derivatization, complete separation among the detected amino acids with acceptable analytical performance can be achieved. Compared to other derivatization reagents, this reagent is simple, commercially available, and should be applicable to a wide range of drugs and compounds containing a secondary or primary amino group.

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