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Optimization of a modified electrode for the sensitive and selective detection of α-dipeptides

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Abstract

Sensitive and selective detection of dipeptides is important in neurochemistry. We have developed a flexible detection scheme for dipeptides based on a modified carbon electrode. The modification arises from the anodic treatment of the carbon electrode in alkaline solution. The flexibility of the detection scheme arises from the different conditions used in both the modification and the detection. It is shown that the modification step requires the presence of cupric ion, while the detection step does not. On the other hand, it is shown that the presence of copper in the detection eluent, as well as the pH of the environment, can be used in controlling the selectivity of the modified electrode. For example, the modified electrode is more selective for α-dipeptides over β- and γ-dipeptides as well as amino acids at pH 9.8, whereas it is selective for all dipeptides over amino acids at pH 8.0. Detection limits of dipeptides on the order of 10 n*M* were achieved at pH 8.0 by flow-injection analysis with a knotted Teflon tubing connecting the injector and the detector that gave a typical peak volume of about 0.50 ml at 1.0 ml/min. From surface analysis it is shown that the oxygenation of the glassy carbon electrode gives rise to the selectivity. The oxidation of dipeptides at the modified electrode is completely inhibited by 10 m*M* Mg^{2+} in the eluent.

1. Introduction

Due to the increased understanding of the role of L-aspartate (Asp) and L-glutamate (Glu) as excitatory amino acids (EAA), research in the area has turned to the search for other endogenous ligands that act on EAA receptors. In particular, some acidic dipeptides such as γ-Glu-Gln have been observed to interact with EAA receptors in vitro [1–4] and in vivo [4,5]. Numerous β-aspartyl dipeptides have been found in human urine [6] but their functions in the central nervous system (CNS) are not clear. The release of glutathione, a γ-glutamyl tripeptide, has been observed during ischemia [7]. Despite all these findings with β- and γ-dipeptides, little analytical work has been done with the most common dipeptides, the α-dipeptides, because of the lack of sensitive and selective detection techniques.

Traditional analytical methods for the detection of peptides are based on UV absorbance of the amide bond, which is too general and has poor sensitivity [8–11], or fluorescence detection following derivatization with reagents such as *o*-phthalaldehyde which are useful in amino acid analysis [8,12–15]. Although quite effective for amino acids, and for β- and γ-dipeptides, the OPA detection technique suffers a severe loss in

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sensitivity for α-dipeptides. Because of its amine specificity, OPA detection of N-acylated compounds such as N-acetyl-Asp-Glu (NAAG), which has no primary amine, is not possible.

Besides sensitivity, an analytical procedure intended for eventual application by neurochemists must have good selectivity for the compounds studied. An example of the need for the selectivity is cysteine sulfinate in the brain. Over time, as analytical methods have improved, the reported concentration of cysteine sulfinate has gone down by four orders of magnitude [16–19].

Previous work on the electrochemical detection of peptides that do not have electroactive functional groups has been done with the biuret reaction [19–21]. In this procedure, the peptide reacts with Cu(II) to form a Cu(II)–peptide complex. This converts previously electroinactive peptides into electroactive species. The oxidation of the Cu(II)–peptide to the Cu(III)–peptide at the upstream anode and the corresponding reduction of Cu(III)–peptide to Cu(II)–peptide at the downstream cathode in a dual series amperometric detector provide for the detection [8]. The reversible Cu(II)/Cu(III) electrochemistry enables the use of the sensitive and selective dual glassy carbon electrode [8,22]. While this detection scheme is useful for peptides containing three or more amino acid residues, it is unfortunately not ideal for dipeptides because the oxidation of the Cu(II)–dipeptide complex requires a high oxidation potential (>0.9 V) [12].

We recently discovered that dipeptides can be detected without the biuret reagent [12]. This involves the use of a modified glassy carbon electrode. The upstream anode becomes modified upon exposure to a high applied potential, ≥ 1.2 V vs. Ag/AgCl. The sensitivity for dipeptides with the modified electrode at a useful analytical anodic potential and pH, i.e. 0.90 V and pH 8.0, is dramatically increased as compared to an unmodified electrode [12]. Both the anodic (modified electrode) and the cathodic (unmodified electrode) signals are useful at pH 8.0 and in the absence of the biuret reagent in the detection eluent. However, it is generally the cathodic signal that is useful at pH >9.5 and in the presence of the biuret reagent in the detection eluent because of the high anodic background. The lowest detection limits are obtained at pH 8.0 and in the absence of the biuret reagent.

There are three objectives of this research. First, the parameters of the modification protocol must be understood in order to optimize conditions with respect to both sensitivity and selectivity. Second, the primary surface chemistry of the modified electrode must be determined to allow intelligent discussion of the surface electrochemistry. Last, we want to demonstrate the applicability of the modified electrode coupled with anion-exchange chromatography for the detection of acidic dipeptides. The biological applications of this detection scheme are underway and will be presented in due course.

2. Experimental

2.1. Chromatographic and detection equipment

A Waters (Milford, MA, USA) 600-MS pump and an ISCO (Lincoln, NE, USA) Model 100DM syringe pump were used to pump the mobile and the post-column phases, respectively. The anodic and cathodic potentials were applied from a BAS (W. Lafayette, IN, USA) LC-4C potentiostat to a BAS dual glassy carbon electrode with a 0.002 inch spacer thickness. All samples were injected into a 20-μl loop using a 100-μl syringe. A Nucleosil 5 SB column (25×4.6 cm, Keystone Scientific, Bellefonte, PA, USA) with guard column was used to separate dipeptides. A knotted Teflon tube (0.95 ml, 90 cm $\times$ 0.58 mm I.D.) in place of the column was used for flow-injection analysis (FIA) to separate the injection spike from the peak. All data were recorded on a chart recorder and through EZChrom software (Scientific Software, San Ramon, CA, USA) installed on a DTK IBM compatible computer. Peak areas were determined after manually specifying the limits of integration for each peak.

All data presented are obtained from a single dual glassy carbon electrode in a serial configura-

tion. The downstream cathode is always set at 0.0 V vs. Ag/AgCl. All applied potentials given below are referenced to Ag/AgCl, 3 *M* NaCl reference electrode (BAS).

2.2. Reagents

ACS certified monobasic and dibasic sodium phosphate (Fisher, Pittsburgh, PA, USA), sodium bicarbonate (Mallinckrodt, Paris, KY, USA), sodium carbonate (J.T. Baker, Phillipsburgh, NJ, USA), magnesium sulfate (Fisher), glacial acetic acid (J.T. Baker), lithium acetate and lithium sulfate (Aldrich, St. Louis, MO, USA), and analytical grade copper sulfate pentahydrate (Fisher) were used without further purification. Sodium potassium tartrate (Fisher) was recrystallized from water before use.

All amino acids, amino acid derivatives, and peptides were purchased from Sigma (St. Louis, MO, USA) except Asp-Asp, Asp-Glu, Glu-Asp, and Glu-Glu (all from Bachem, King of Prussia, PA, USA). β-Asp-Tau was synthesized after Ienaga et al. [23].

One-letter shorthand for amino acids is used in figure legends and three-letter ones are used in the text. The one-letter abbreviations are: A = Ala, C = Cys, D = Asp, E = Glu, G = Gly, and V = Val.

2.3. Mobile phases and post-column phases

Several buffers were used. The pH 8.0 phosphate buffer (0.1 *M* for FIA and 0.5 *M* for HPLC) consisted of dibasic and monobasic sodium phosphate in a 95:5 molar ratio with sodium bicarbonate at a concentration of 10% of the total phosphate concentration. The 0.2 *M* pH 9.8 carbonate buffer was made from equimolar amounts of bicarbonate and carbonate as sodium salts. The 5.0 m*M* pH 4.6 acetate buffer used as mobile phase for HPLC was made from equimolar amounts of lithium acetate and glacial acetic acid with varying amounts of lithium sulfate. Biuret reagents were prepared in each of the basic buffers. The stock solutions of tartrate (0.3 *M*) and copper sulfate (0.1 *M*) are mixed first, then this solution is mixed with the buffer solution before diluting to the appropriate volume. In this way, copper(II) will not precipitate. The concentrations of copper sulfate and tartrate are 0.1 m*M* and 0.3 m*M*, respectively, in all of the reagent formulations, unless otherwise indicated.

All mobile and post-column phases were dissolved in or diluted with twice-deionized water and vacuum filtered through 0.45-μm type HA filters (Millipore Corp.). The solutions were then purged with helium gas to remove oxygen.

2.4. Electrode modification protocol

The following steps comprise the protocol used for glassy carbon anode modification:

(1) Polish the electrode with 0.05 μm γ-alumina (LECO, St. Joseph, MI, USA) on polishing cloth (BAS).

(2) Vigorously rinse the electrode surface with twice-deionized water.

(3) Ultrasonicate the electrode with 50 ml twice-deionized water twice for 5 min each time.

(4) Assemble the detector cell and place it in the flow stream.

(5) Modify the anode with an applied potential of 1.2 V or 1.4 V vs. Ag/AgCl at a buffer flow-rate of 1.0 ml/min for a given time.

Such a modified electrode can be used for dipeptide detection up to weeks without retreating the electrode surface, and there is no loss of sensitivity observed for up to weeks. During overnight periods the electrode is left open to ambient air without any special storage. The cathode was not modified.

3. Results and discussion

3.1. Optimization of the modified electrode

The parameters that control the modified electrode's performance include the presence of the biuret reagent in solution, the modification and detection pH, and the modification time and potential [12]. The effect of these parameters will be discussed below.

Biuret reagent

In this study, we intend to investigate the effect of copper and tartrate in the modification protocol. We have modified the glassy carbon anode in the presence and absence of copper and tartrate and used both the modified anode and the unmodified cathode (downstream) for dipeptide detection. Table 1 gives the results obtained from flow-injection analysis (FIA) using the modified and the unmodified electrodes for a series of α-, β-, and γ-dipeptides. The sensitivities are corrected for the blank injection. Each number is the mean value from two or three replicate injections. The pooled standard deviation of the areas obtained from each electrode modification is given in the first row of the table. Sensitivities that are less than twice the pooled standard deviation are in parentheses. Negative sensitivities are data in which the peak area is larger in the blank than in the presence of an analyte. The magnitudes of the standard deviations indicate that most of these negative signals are real. We speculate that the negative signals are from a suppression of the ambient background current. The background current is suppressed in the presence of certain peptides because they adsorb and alter the electroxidation of solvent/impurities. The adsorption is reversible.

The data clearly show that the modification requires the biuret reagent. In the absence of the biuret reagent in the detection eluent, the sensitivities of the anode modified in the presence of Cu^{2+} and tartrate are increased as compared to sensitivities obtained from an unmodified and a modified-without-Cu^{2+} and tartrate electrode. Note that experiments cannot be done with Cu^{2+} alone.

Once we established that the modification needed the biuret reagent, we went on to test the detection in the presence and in the absence of the biuret reagent. The results, also obtained

Table 1
Effect of Cu^{2+} on the modification of the anode at pH 9.8

	Cathodic sensitivity (nC/pmol), anode 0.90 V, cathode 0.0 V[a]					
	Unmodified electrode		Modified without Cu^{2+}		Modified with 0.1 mM Cu^{2+}	
	Anode	Cathode	Anode	Cathode	Anode	Cathode
Pool S.D., σ_p	0.036	0.001	0.068	0.004	0.120	0.004
α-Asp-Gly	0.202	0.037	0.163	0.034	0.787	0.130
α-Asp-Val	(−0.062)[b]	0.028	−2.98	−0.048	0.476	0.094
α-Ala-Gly	0.406	0.119	−1.93	0.035	1.35	0.260
α-Ala-Ala	0.696	0.173	−1.92	0.078	1.54	0.340
Gly-Gly	0.210	0.048	−2.46	−0.035	(0.139)[b]	0.058
α-Asp-Asp	0.137	0.003	−2.67	−0.072	1.39	0.012
α-Glu-Glu	(−0.235)[b]	0.013	−2.66	−0.052	0.250	0.050
α-Asp-Glu	−0.199	0.016	−2.80	−0.052	(0.026)[b]	0.067
α-Glu-Asp	(−0.067)[b]	(0.001)	−2.37	−0.073	0.790	−0.066
β-Asp-Gly	0.074	0.044	1.98	0.064	2.021	0.093
β-Asp-Val	−0.077	0.014	−2.79	−0.053	0.636	0.036
β-Ala-Gly	0.345	0.020	−1.44	−0.055	1.103	0.039
β-Ala-Ala	0.094	0.012	−2.69	−0.054	0.560	0.021
γ-Glu-Gly	−0.273	0.005	−2.98	−0.059	(−0.007)[b]	(0.007)[b]
γ-Glu-Val	−0.267	0.004	−3.20	−0.067	(−0.182)[b]	0.005

[a] Modification: 0.2 M pH 9.8 carbonate buffer with and without the biuret reagent, 1.4 V for 4 h at 1.00 ml/min. Detection: 0.2 M pH 9.8 carbonate buffer without the biuret reagent.

[b] Signal magnitude $< 2 \times$ pooled standard deviation, σ_p.

Table 2
Effect of Cu^{2+} on the detection at pH 9.8

Compound	Cathodic sensitivity (nC/pmol), anode at 0.85 V[a]	
	Detected without Cu^{2+}	Detected with Cu^{2+}
Asp	0.009	0.000
α-Asp-Gly	0.100	0.042
β-Asp-Gly	0.075	0.028
γ-Glu-Gly	0.001	0.033
Ala-Ala-Ala	0.570	0.590

[a] Modification: 0.2 *M* pH 9.8 carbonate buffer with the biuret reagent. Detection: 0.2 *M* pH 9.8 carbonate buffer with and without the biuret reagent.

from FIA, for a representative amino acid, α-, β-, and γ-dipeptides, and a tripeptide are shown in Table 2. Note that the tripeptide Ala-Ala-Ala exists as the reversible electroactive biuret complex in Cu(II) containing solution. There are two conclusions to be drawn from the data. First, there is no need for copper in the detection phase once the electrode is modified in the presence of copper. Second, it is more selective to detect α- and β-dipeptides over γ-dipeptides without copper but more selective to detect all dipeptides over amino acids in the presence of copper. This point will become more convincing when results from a larger pool of samples are presented below. Then, we conclude that the biuret reagent is important for the modification and optional during operation for the control of selectivity.

Eluent pH

Four different FIA experiments were performed to determine the effect of pH on the modification and detection. Electrodes were modified at pH 8.0 and pH 9.8, with the biuret reagent at 1.2 V for 4 h at a flow-rate of 1.0 ml/min. These modified electrodes were then tested individually with both pH 8.0 and pH 9.8 eluents which also contained the biuret reagent.

From the results (not shown) from the two experiments in which the electrode is modified in one pH and used for detection at the other pH, we concluded that it is only the detection pH that makes a difference, not the modification pH. Figs. 1 (pH 9.8) and 2 (pH 8.0) show the cathodic sensitivity (cathode at 0.0 V) obtained when the anode is set at 0.8 V, 0.9 V and 1.0 V. The mobile phases are 0.1 *M* pH 9.8 carbonate buffer and 0.2 *M* pH 8.0 phosphate buffer, both with 0.1 m*M* $CuSO_4$ and 0.3 m*M* tartrate. Note that each cluster of three sensitivities is a coarse voltammogram. The negative sensitivities, in this case, are attributed to two different factors; one is the adsorption of amino acids or peptides onto

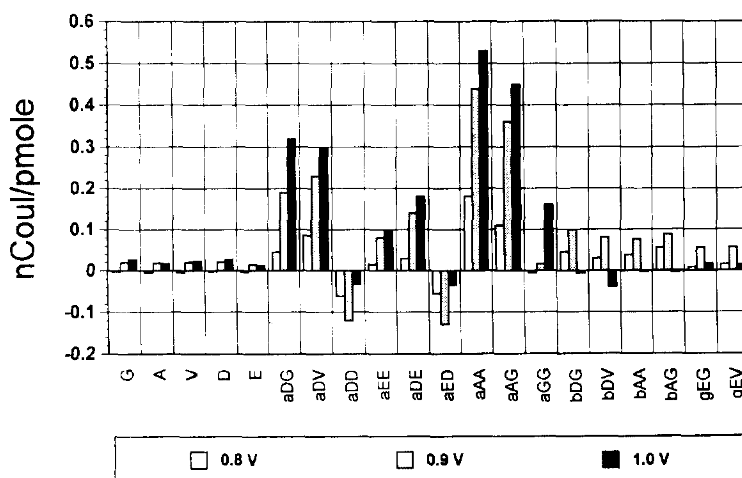

Fig. 1. Cathodic signals with the anode at 1.0, 0.9, and 0.8 V for pH 9.8. Both the modification and the detection are with the same buffer and with the biuret reagent. Modification was done at 1.4 V for 4 h at 1.0 ml/min (a = α, b = β, g = γ).

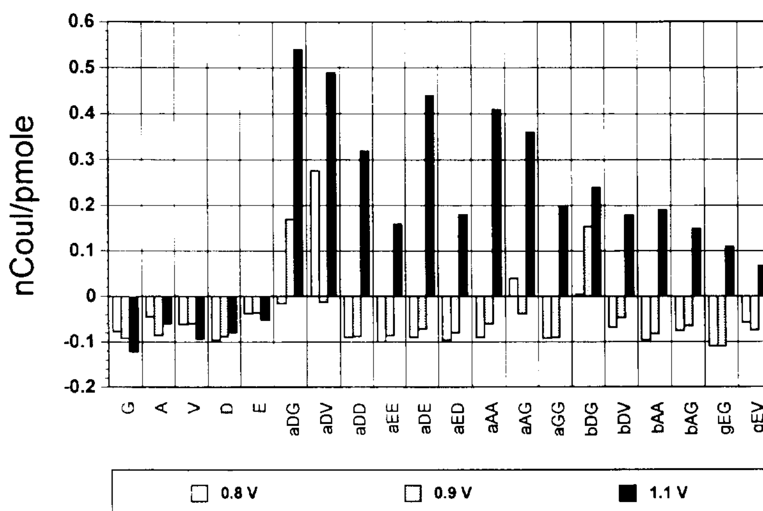

Fig. 2. Cathodic signals with the anode at 1.0, 0.9, and 0.8 V for pH 8.0. Both the modification and the detection are with the same buffer with the biuret reagent. Modification was done at 1.4 V for 4 h at 1.0 ml/min (a = α, b = β, g = γ).

the modified electrode surface as discussed before, and the other is the uptake of copper by the solutes. The onset potentials for significant background current from the Cu(II)–tartrate complex are 0.75 and 0.65 V at pH 8.0 and 10.0, respectively. If the analyte forms the Cu(II)–peptide complex and the oxidation of that complex has a higher onset potential than the Cu(II)–tartrate complex, a negative sensitivity results because of the lowering of the background signal. However, the full mechanistic aspect of this issue is still awaiting to be uncovered. To return to the figures, by comparing the two graphs with the last column of Table 1 (data obtained with an anodic potential of 0.9 V), a few conclusions are clear. In the presence of copper in the detection eluent, the modified electrode is selective for all dipeptides over amino acids at pH 8.0 with an anodic applied potential of $\geq$ 1.1 V (detecting at the cathode), but it is more selective for α-dipeptides over other dipeptides and amino acids at pH 9.8 with an applied anodic potential of $\geq$ 1.0 V. Furthermore, the modified anode becomes useful without the copper in the detection eluent, as concluded from comparison of the 0.9 V data of Table 1 and Fig. 1. We conclude in this section that the detection eluent pH and the biuret reagent are important and flexible factors in controlling the selectivity of dipeptide detection.

Modification potential and time

Table 3 gives the anodic signal at 0.85 V for different modification potentials and time at pH 8.0. The electrode is modified with a 0.1 m*M* copper and 0.3 m*M* tartrate in 0.2 *M* pH 8.0 phosphate buffer. The detection is carried out with the same solution. The data show that the increase in signal reaches a saturation point at 1.2 V for 4 h.

We conclude, in this section, that the best modification is done with a pH 8.0 buffer in the presence of copper at 1.2 V for 4 h. The detection buffer pH and the presence and absence of the biuret reagent are factors for controlling the selectivity of the system.

3.2. *Hydrodynamic voltammogram by FIA*

Figs. 3–5 show the hydrodynamic voltammograms (HDV) obtained by FIA with and without the modified electrode at pH 8.0 and pH 9.8 in the presence of 0.1 m*M* copper. Data plotted at potentials less than 0.4 V are the cathodic signal

Table 3
Effect of modification time and potential: anodic sensitivity (nC/pmol) at 0.85 V[a]

Compound	1.2 V			1.4 V		
	0 h	4 h	11 h	0 h	4 h	11 h
Asp	−0.003	−0.027	−0.074	−0.0005	0.000	−0.050
α-Asp-Gly	0.008	0.160	0.180	0.005	0.170	0.180
β-Asp-Gly	0.010	0.310	0.300	0.004	0.330	0.310
γ-Glu-Gly	−0.005	0.006	0.021	−0.002	0.004	−0.019
Ala-Ala-Ala	0.710	0.910	0.730	0.690	0.920	0.840

[a] Modification: 0.1 M pH 8.0 phosphate buffer with biuret reagents at 1.0 ml/min. Detection: same eluent.

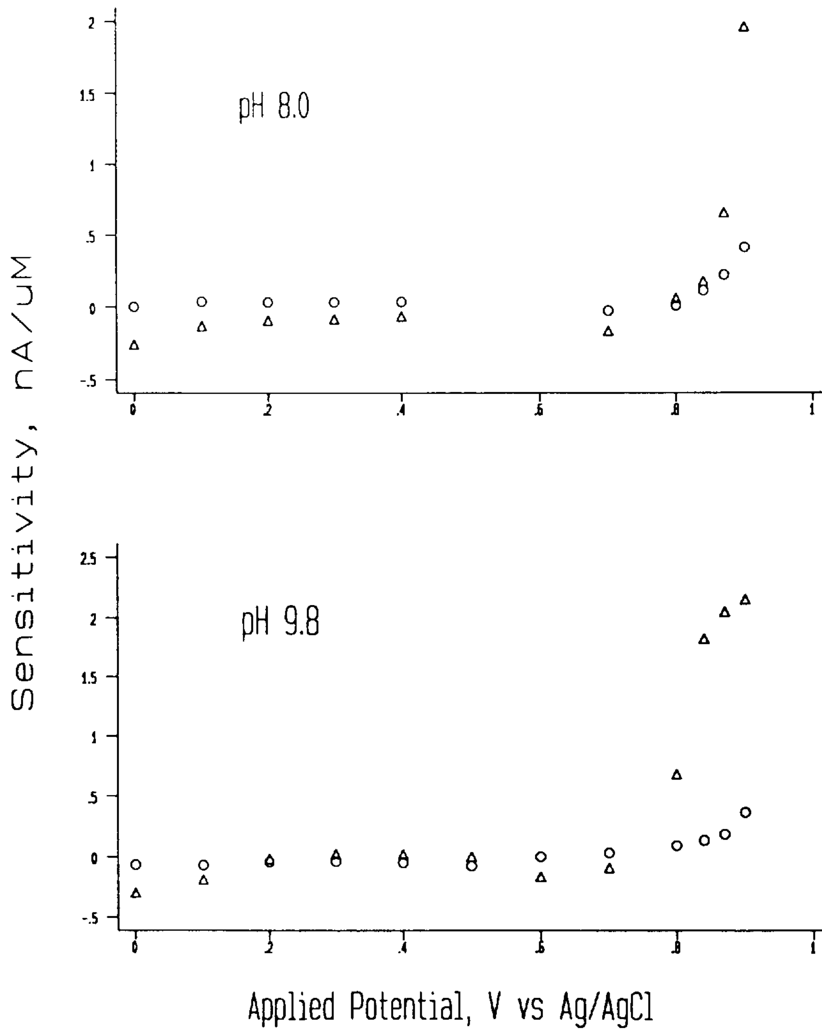

Fig. 3. Hydrodynamic voltammogram of α-Asp-Gly at both pH 8.0 and 9.8. Electrodes were modified in their respective buffers with the biuret reagent and detected with the same eluent: △ = modified electrode, and ○ = unmodified electrode.

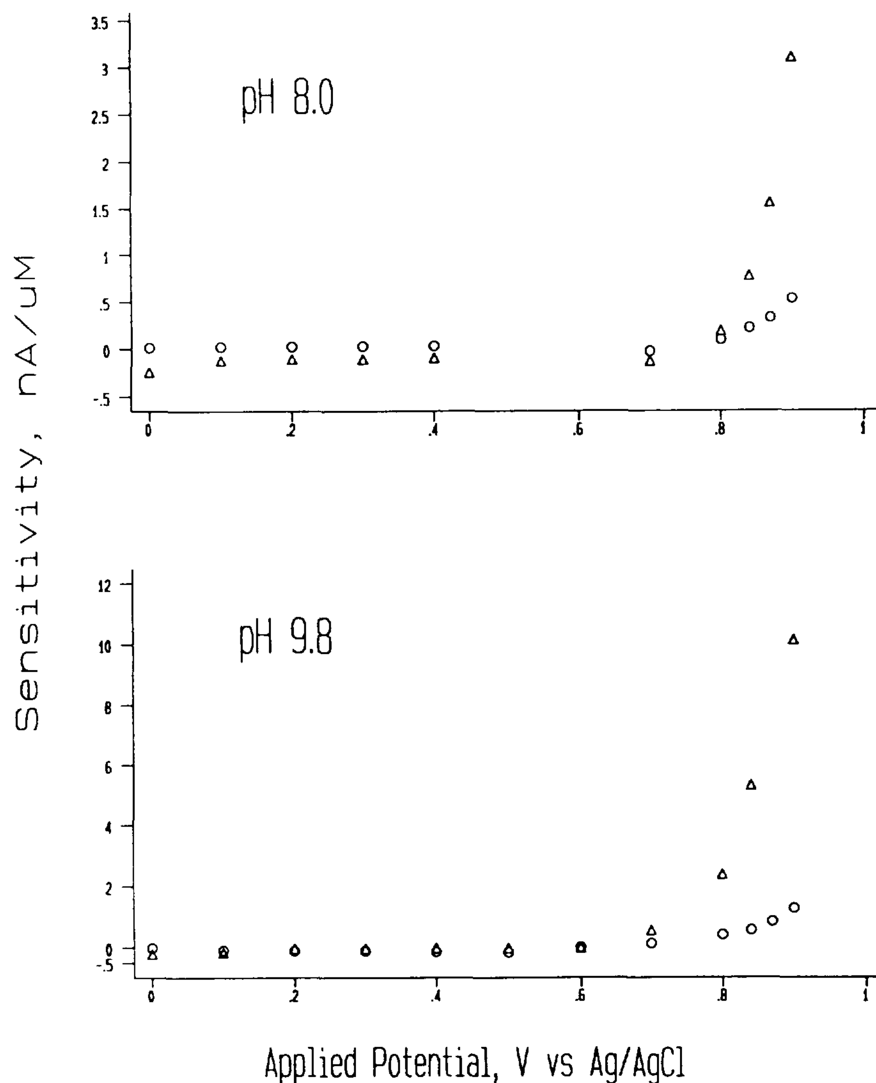

Fig. 4. Hydrodynamic voltammogram of β-Asp-Gly at both pH 8.0 and 9.8. Electrodes were modified in their respective buffers with the biuret reagent and detected with the same eluent; △ = modified electrode, and ○ = unmodified electrode.

(0 to 0.4 V) with the anode at 0.90 V. The remaining data are anode sensitivity. Comparing the modified with the unmodified electrode results reveals a dramatic shift in the onset potential of the signal-generating species upon modification. The control compound, Ala-Ala-Ala, is not affected as much by the modification as the dipeptides.

3.3. Detection limit by FIA

Fig. 6 shows the graph of the inverse of the detection limit in nM^{-1} obtained by FIA for both pH 8.0 and pH 9.8 using the cathodic signals with the anodes at 1.1 and 1.0 V, respectively. The detection limit is defined as the concentration that would give 3/5 of the peak-to-

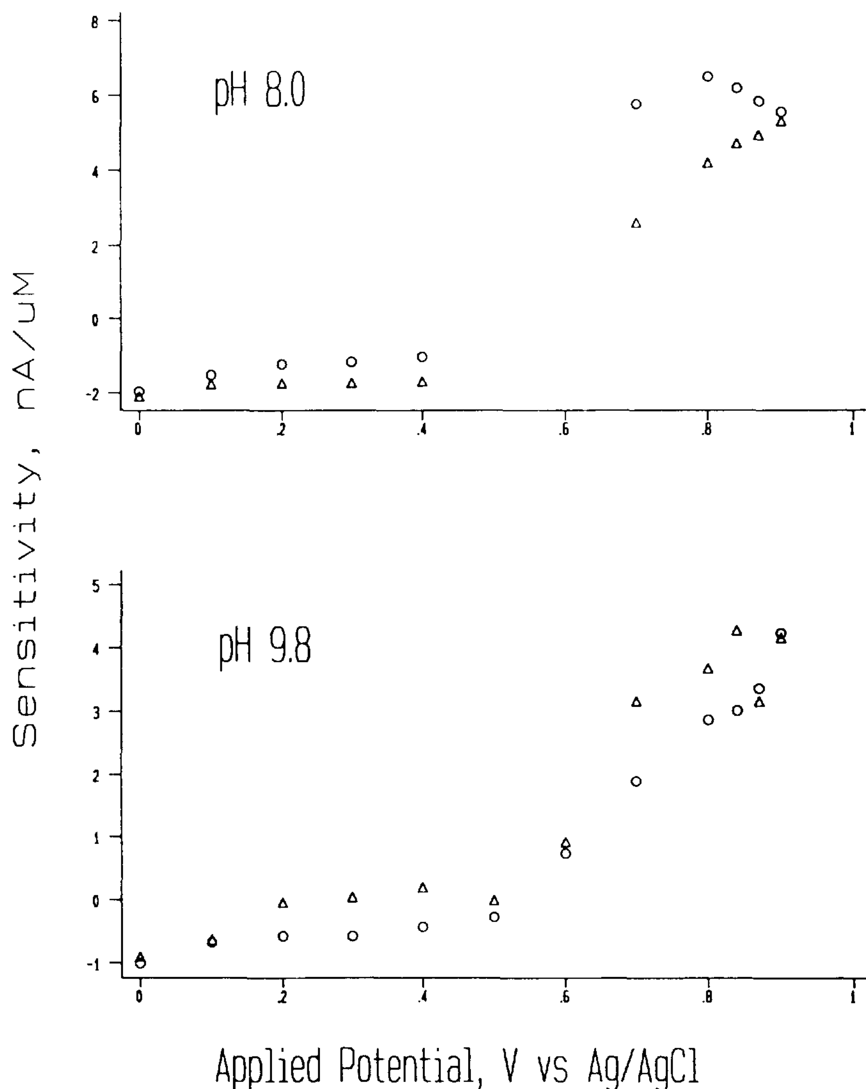

Fig. 5. Hydrodynamic voltammogram of Ala-Ala-Ala at both pH 8.0 and 9.8. Electrodes were modified in their respective buffers with the biuret reagent and detected with the same eluent; △ = modified electrode, and ○ = unmodified electrode.

peak noise which we take to be approximately five times the standard deviation of the baseline. As evidenced by the graph and the discussion above, both the detection limit and the selectivity of dipeptides over amino acids are by far superior with the pH 8.0 buffer. However, selective detection of α-dipeptides over other dipeptides is better with the pH 9.8 buffer. Lastly, we should point out that nM detection limits are possible for the dipeptides.

3.4. Surface chemistry

We pointed out in the last few paragraphs that the modification protocol requires copper but it is not necessarily needed for the detection. One

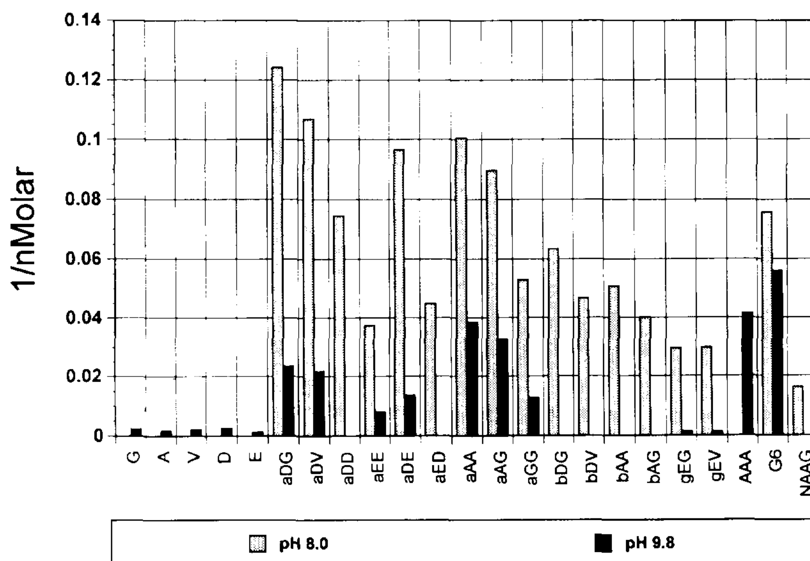

Fig. 6. Inverse of the detection limit plot, obtained at both pH 8.0 and 9.8. Both modification and detection were done in the presence of the biuret reagent (a = α, b = β, g = γ).

might expect some kind of copper salt to have been deposited on the electrode surface during modification that provides the amplification of the signal. After all, a copper electrode has been used to detect amino acids [24]. In answering this question, we turned to spectroscopy for surface analysis. Results of the ESCA spectra of the modified (bottom) and the unmodified (top) glassy carbon electrode surfaces are shown in Fig. 7. The modification was carried out in a polyethylene container with constant stirring with a pH 9.8 carbonate buffer at 1.4 V for 4 h. The control electrode was put in the same solution but without the applied potential. Modification was observed visually by the colored appearance of the surface when observed at an angle. The surprising result is that the expected copper band is not present, rather there is only a change in the O/C ratio and a silicate band that probably originated from the glass reference electrode used. The O/C ratio changes from the expected 0.31 [25] to 0.53. Oxygenation of the carbon surface has been carried out before with much higher potentials, i.e. 1.8 V [25]. The same spectroscopic results were observed with a pH 8.0 modification.

Armstrong et al. [25], studying cytochrome c voltammetry with a pyrolytic carbon electrode at pH 8.0, discovered that the addition of magnesium ion to the electrolyte solution decreased the cytochrome c signal. They attributed this to the Mg^{2+} binding to surface oxygen.

To test the importance of oxygen on the modified electrode surface, we used $MgSO_4$ in the eluent with an electrode that had been modified with a pH 8.0 buffer and biuret reagents. The results obtained with a pH 8.0 detection eluent with various amounts of Mg^{2+} added are shown in Fig. 8 for the analyte β-Asp-Gly. The data clearly demonstrate that the surface oxygen is involved in the oxidation of dipeptides.

3.5. Linearity of the modified electrode response

Fig. 9 shows the calibration curves of β-Asp-Gly obtained with a pH 8.0 modification and detected with the same eluent. Fig. 9A gives the cathodic sensitivity when the anode is at 0.85 V and 1.10 V. Good linearity was observed with the cathode (both with $r^2 > 0.99$). With the anodic signal at 0.80 and an extended concentration

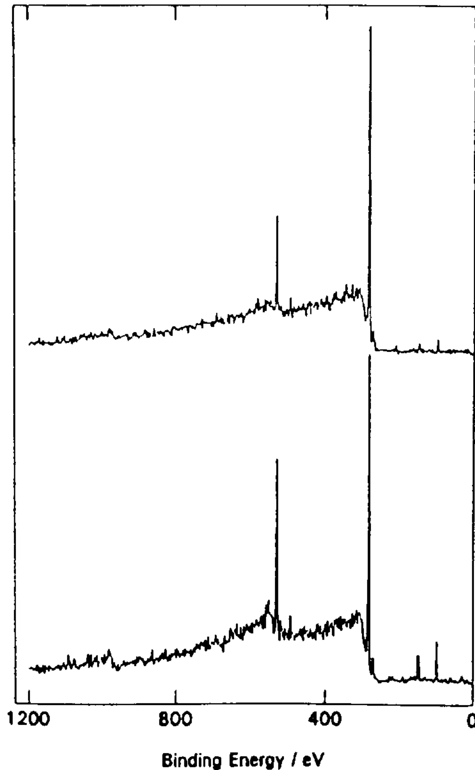

Fig. 7. ESCA spectra of the modified and the unmodified glassy carbon surface. (Top) Unmodified, and (bottom) modified, at pH 9.8 with the biuret reagent for 4 h at 1.4 V with constant stirring of the modification buffer.

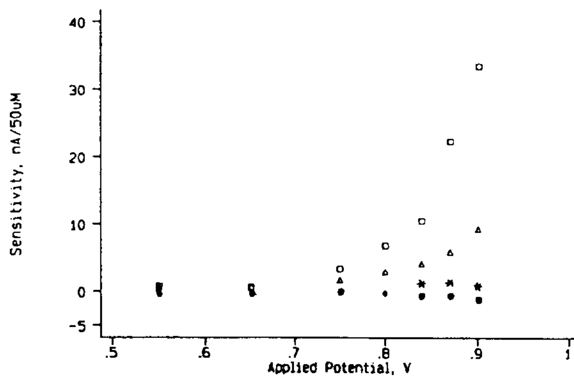

Fig. 8. Effect of Mg^{2+} on the modified GCE surface: □ = without $MgSO_4$, △ = with 3.0 mM $MgSO_4$, * = with 10 mM $MgSO_4$, and ● = no modification and without $MgSO_4$.

range, however, a curve suggestive of adsorption of peptide onto the modified electrode becomes more clear, as shown in Fig. 9B. These observations support our speculation mentioned previously that these peptides are adsorbed onto the modified electrode surface.

3.6. Example of chromatograms

Fig. 10 shows the chromatogram obtained with an electrode modified under the optimum conditions described above for dipeptides, pH 8.0 buffer with 0.1 mM Cu^{2+}, at 1.2 V for 4 h at a flow-rate of 1.0 ml/min. The separation was done with a Nucleosil SB anion-exchange column eluted with a 5 mM pH 4.6 acetate buffer and a step gradient from 1.5 mM to 15 mM Li_2SO_4 after the first 20 min. The post-column phase used is a 0.5 M pH 8.25 phosphate buffer with a mixing ratio of 2:1, mobile phase to post-column phase. Thus, there is no Cu^{2+} in the flow stream. An amount of 1 nmole of each of eight dipeptides and glutathione was injected, four α-, two β-, and two γ-dipeptides. In general, the detector under these conditions is much more sensitive for α- than β- than for γ-dipeptides. For example, note that there is no peak for glutathione, a γ-glutamyl tripeptide that is expected to be present in the highest concentration in biological samples.

As discussed before, the best signal-to-noise (S/N) ratio is obtained in the absence of the biuret reagent in the detection eluent, but the most selective detection for α-dipeptides over β-dipeptides, γ-dipeptides, and amino acids is obtained in the presence of the biuret reagent. The anodic signal is useful only in the absence of biuret reagent. The chromatogram shown clearly demonstrates these points. The cathodic signal in this case, however, exhibits negatively tailing peaks and would not be as useful as the anodic signal. On the other hand, with the presence of the biuret reagent, the cathodic peaks are symmetrical, have the best S/N ratio, and will be the choice for the detection. Since the surface chemistry of the modified anode is not fully understood, an explanation for the negative tailing of the downstream cathodic signals in the

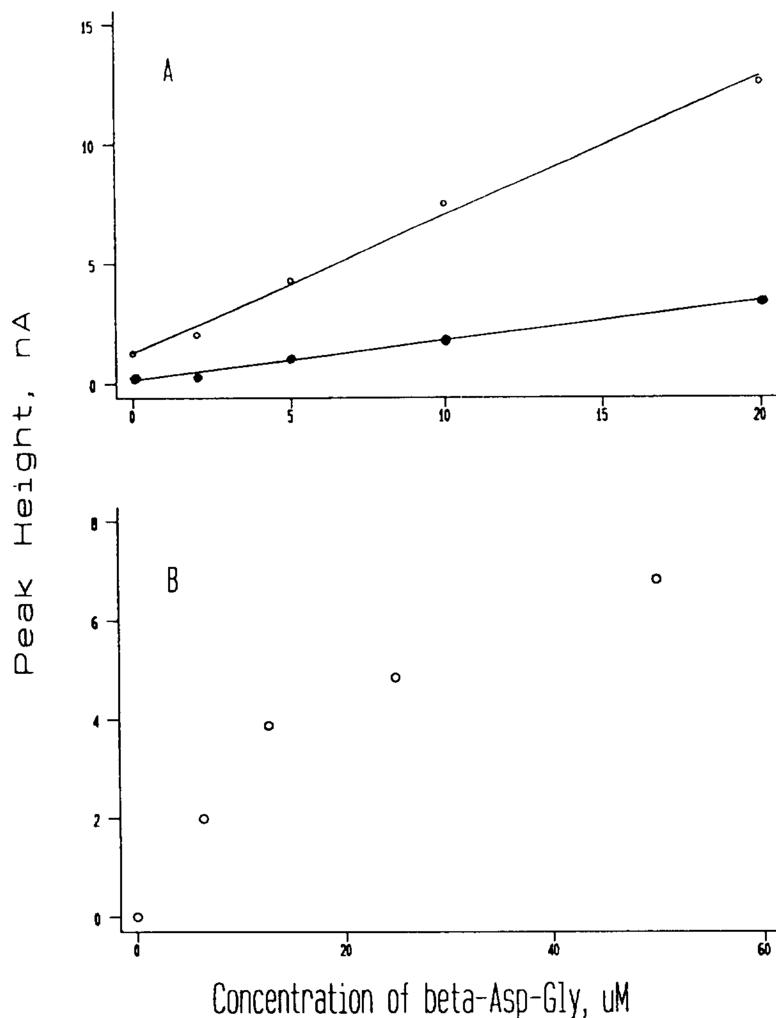

Fig. 9. Calibration curves of β-Asp-Gly with a pH 8.0 modification, cathodic signal. (A) Anode at 1.10 V (○) and at 0.85 V (●). (B) Anodic signal at 0.8 V.

absence of the biuret reagent is not attempted. However, it will be addressed when the modified electrode surface is probed in full detail by microscopy in the near future.

4. Conclusions

We have demonstrated that the modified electrode works for the sensitive and selective detection of dipeptides. The best modification is carried out at pH 8.0 eluent in the presence of the biuret reagent with an applied anodic potential of 1.2 V for 4 h at a flow-rate of 1.0 ml/min. Selectivity can be altered by means of controlling both the pH of the detection eluent and the presence and the absence of the biuret reagent. Surface analysis shows that it is the oxygenation, in the presence of the copper ion during the modification, that is responsible for the selective detection. The detection system exhibits nM detection limits. Future work will be

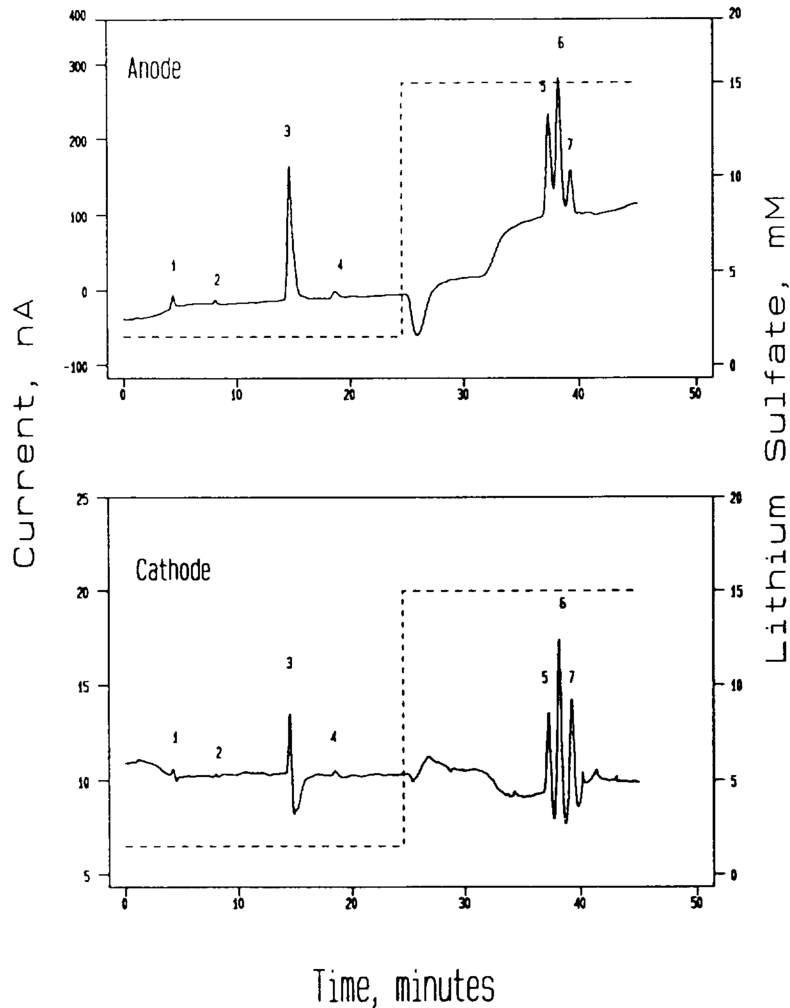

Fig. 10. Chromatograms of 1.0 nmole of eight acidic dipeptides and glutathione obtained with a pH 8.0 modified electrode. Mobile phase: 5.0 mM pH 4.6 acetate buffer with a step gradient of Li_2SO_4 as shown by the dotted line at 0.8 ml/min. Post-column phase: 0.5 M phosphate–acetic acid pH 8.25 buffer at 0.4 ml/min. Applied potentials are 1.1 V and 0.0 V for the anode and the cathode, respectively. Peaks: 1 = excess acetate, 2 = β-Asp-Tau, 3 = β-Asp-Gly, 4 = γ-Glu-Cys, 5 = α-Asp-Asp, 6 = α-Glu-Asp and α-Asp-Glu, 7 = α-Glu-Glu. No peak was observed for glutathione. See text for more details.

focused on the biological applications of the system.

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