

Investigations of the Potential-Dependent Structure of Phenylalanine on the Glassy Carbon Electrode by Infrared–Visible Sum Frequency Generation

Joonyeong Kim, Keng C. Chou, and Gabor A. Somorjai*

Department of Chemistry, University of California at Berkeley, Berkeley, California 94720, and
Materials Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720

Received: April 1, 2002; In Final Form: June 4, 2002

We investigated the structure of adsorbed L-phenylalanine (Phe) on the glassy carbon electrode (GCE) as a function of electrode potential using infrared-visible sum frequency generation (SFG) vibrational spectroscopy. Experiments using partially deuterated Phe confirmed that SFG signal originates from the CH stretch modes of the methylene group (CH₂) in Phe. Quantitative analysis of SFG spectra revealed that Phe molecules adopt a conformation in which the dipole contribution of the asymmetric mode of the methylene group becomes greater than the symmetric mode with respect to the surface normal at highly positive electrode potential.

Interactions of specific amino acid sequences within a protein are known to affect the overall adsorption process to a solid substrate.^{1,2} For this reason, considerable efforts have been made to understand the adsorption properties of amino acids using various substrates and diagnostic tools. Studies using titanium oxide (TiO₂),^{3,4} silica,^{5,6} alumina,⁵ graphite,⁵ gold,^{4,7} stainless steel,⁸ platinum,^{9,10} and silver¹¹ have consistently shown that the macroscopic adsorption behavior of various amino acids is intimately related to pH, concentration and charges on the amino acids, and the particular solid substrates used. In particular, in situ Fourier transform infrared (FTIR) studies combined with electrochemical techniques have produced valuable information regarding the structures and oxidation mechanisms of adsorbed amino acids at the platinum and gold electrodes.^{7,9,10} FTIR spectra have shown that amino acids are adsorbed by 2-fold coordination via a carboxylate group (CO₂[−]) on the metal surface. Oxidation occurs by producing cyanide, carbon monoxide, carbon dioxide, and other byproducts at elevated electrode potential. Despite this progress, obtaining detailed structural information of adsorbed molecules at the solid/liquid interface has presented a substantial experimental challenge.

For the past decade, infrared-visible sum frequency generation (SFG), a surface-specific vibrational spectroscopy, has emerged as a viable technique for obtaining structural information concerning adsorbed molecules at a number of interfaces and surfaces even in the presence of an overwhelming bulk contribution.^{12–17} In this letter, we report SFG data on the adsorbed amino acid, L-phenylalanine (Phe, see the structure in the inset of Figure 1) at the glassy carbon electrode (GCE)/electrolyte interface as a function of electrode potential. The results showed that adsorbed Phe molecules undergo structural rearrangement by changing electrode potential.

The theory and experimental setup of SFG has been described in detail elsewhere.^{18,19} Briefly, SFG is a second-order nonlinear optical process in which two input lasers with frequencies ω_{ir} and ω_{vis} overlap at an interface to generate an output at the sum frequency, ω_{sfg} . The intensity of the SFG signal, I_{sfg} , is proportional to the square of the surface nonlinear susceptibility, $\chi_s^{(2)}$:

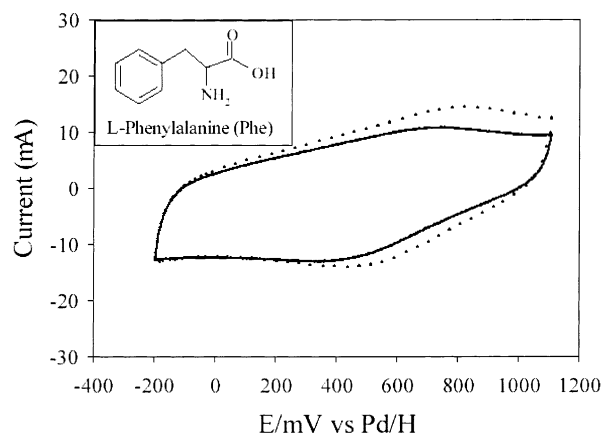


Figure 1. Cyclic voltammetry curves of GCE electrode in 0.1 M LiClO₄ (solid line) and 0.1 M LiClO₄ with 0.01 M Phe (dotted line). Scan speed is 50 mV/s. The inset shows the structure of L-phenylalanine (Phe).

$$I_{\text{sfg}} \approx |\chi_s^{(2)}|^2 I_{\text{vis}} I_{\text{ir}} = |\chi_{\text{NR}}^{(2)} e^{i\delta\nu} + \sum_{\nu} A_{\nu} / (\omega_{\nu} - \omega_{\text{ir}} - i\Gamma_{\nu})|^2 I_{\text{vis}} I_{\text{ir}} \quad (1)$$

$$A_{\nu, IJK} = n \sum_{ijk} \langle (\hat{\mathbf{i}} \cdot \hat{\mathbf{i}})(\hat{\mathbf{j}} \cdot \hat{\mathbf{j}})(\hat{\mathbf{k}} \cdot \hat{\mathbf{k}}) \rangle \beta_{\nu, ijk} \quad (2)$$

where $\chi_{\text{NR}}^{(2)}$, $e^{i\delta\nu}$, A_{ν} , ω_{ν} , and Γ_{ν} denote nonresonant contribution, phase factor, oscillator strength, resonant frequency, and damping constant of the ν th resonant mode, respectively. The oscillator strength, A_{ν} , is related to the hyperpolarizability of the vibrational resonance, β_{ν} , by the number density of contributing oscillators, n , and an orientation averaged coordinate transformation where I, J, K refers to the laboratory-fixed x, y, z coordinates and i, j, k refers to the internal a, b, c coordinates of the oscillator.

SFG spectra in this paper were obtained by the use of a thin-layer electrochemical cell equipped with reference and counter electrodes described in detail elsewhere.¹⁴ To reduce possible interference of the OH and CH stretch modes,²⁰ all SFG experiments were conducted using solutions prepared in deuterium oxide. Details of the laser system, sample preparation,

* To whom correspondence should be addressed. Tel: 510-642-4053. Fax: 510-643-9668. E-mail: somorjai@socrates.berkeley.edu.

and data collection procedures are provided in the Supporting Information. All SFG spectra presented in this paper were collected with the s_{sfg} , s_{vis} , and p_{ir} polarization combination (ssp).

For the SFG and electrochemical studies presented here, we have used a glassy carbon electrode (GCE) as the substrate. GCE has been widely used for the adsorption of various molecules such as nucleic acids and proteins because of its inert mechanical and electrochemical properties.^{21–24} Structurally, GCE consists of randomly oriented carbon atoms with both sp^2 and sp^3 hybridization.²⁵ Atomic force microscopy (AFM) images showed that surface of GCE is nearly flat with a root-mean-square roughness of 1.6 nm over a $20 \times 20 \mu\text{m}^2$ area after polishing. Preliminary studies showed that bare GCE produces a constant nonresonant SFG signal intensity in the range of $2750\text{--}3100 \text{ cm}^{-1}$. Although gold and indium tin oxide (ITO) are preferred due to their well-established adsorption behavior and electrochemical properties, their SFG spectra are more difficult to interpret because of an abnormally large nonresonant background. The magnitudes of the nonresonant SFG signal from ITO and gold are about 40 and 20 times stronger than that from the GCE, giving rise to SFG spectra with no distinguishable CH peaks (data are provided in the Supporting Information). Although platinum exhibits a weak nonresonant background, adsorbed amino acids are known to be easily oxidized even at lower electrode potential.^{9,10}

Figure 1 shows cyclic voltammetry (CV) curves using a GCE electrode in 0.1 M LiClO_4 with and without 0.01 M Phe in the range of -200 and 1100 mV . Both CV curves are nearly comparable and contain broad and weak reversible peaks at slightly different potential values. It is generally believed that the presence of the weak peak is associated with the adsorption and desorption of inorganic anions such as ClO_4^- at a positive potential.²⁶ The two CV curves nearly overlap below 0.0 mV indicating that adsorption of Phe on the GCE begins around zero potential with respect to the Pd/H electrode. No observable oxidation–reduction peaks of Phe are observed over the potential range investigated. This result is in contrast to the reported CV curves collected on gold electrodes in which peaks for adsorption and subsequent oxidation of Phe have been observed.⁷

Figure 2 shows SFG spectra at the GCE/electrolyte interface with 0.01 M Phe as a function of electrode potential. All SFG spectra contain two negative peaks around 2855 and 2935 cm^{-1} with varying magnitude. The peak shape in the SFG spectra is attributed to the relative phase difference between the SFG signals from adsorbed Phe and the nonresonant signal from GCE ($e^{i\phi_{\text{nv}}}$ in eq 1).¹⁷ Similar peak shapes in SFG spectra are also observed from molecules adsorbed on other solid substrates.^{16,17}

To determine the structure of adsorbed Phe, it is crucial to identify CH bonds that are responsible for the SFG spectra shown in Figure 2. To deconvolute the contributions of the methylene (CH_2), the CH group at the chiral center, and the CH groups in the phenyl ring to the SFG spectra, we performed experiments using Phe- d_2 in which two hydrogen atoms in the methylene group are deuterated (98%, Isotec Inc.). The results showed that SFG spectra no longer contain vibrational features indicating that the CH stretch modes observed in Figure 2 originate from the methylene group, CH_2 (data are provided in the Supporting Information). Therefore, peaks at 2855 and 2935 cm^{-1} can be assigned to the symmetric CH_2 (CH_2 (s)) and the asymmetric CH_2 (CH_2 (a)) stretch modes for the methylene group, respectively.^{27,28}

As the isoelectric point of Phe is 5.75, most Phe molecules exist in the zwitterionic form for the experimental conditions

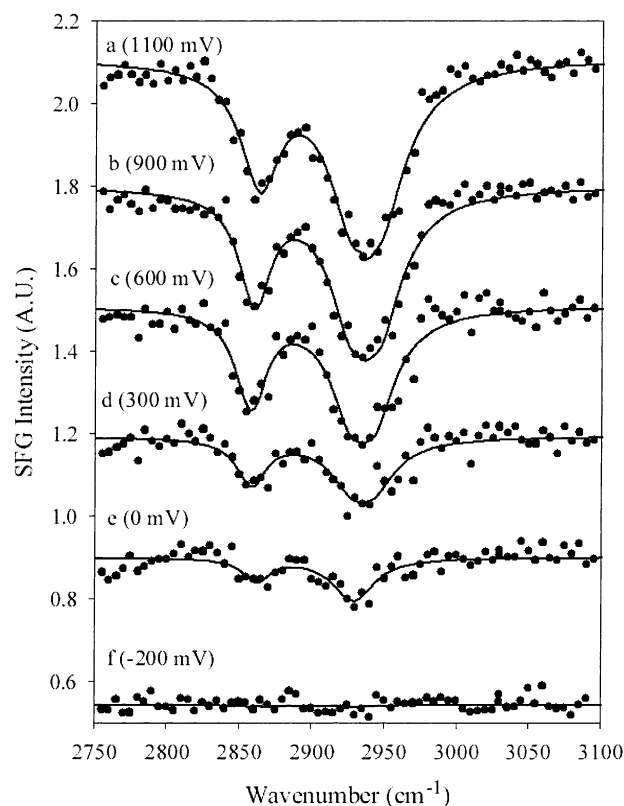


Figure 2. SFG spectra in the CH stretch region of the GCE/water interface with various electrode potentials 1100 (a), 900 (b), 600 (c), 300 (d), 0 (e), and -200 mV (f). Filled circles are collected data and solid lines are fitted data using eq 1. Spectra a, b, c, d, and e were offset from a by 1.5, 1.2, 0.9, 0.6, and 0.3 units of intensity, respectively.

employed here.²⁹ We assume that adsorption of Phe is achieved via the interaction between the negatively charged carboxylate group, CO_2^- , and the positively charged GCE as found for other small molecules and amino acids.²⁴ In general, simultaneous observation of the CH_2 (a) and the CH_2 (s) modes for a given experimental polarization combination (ssp) suggests that the dipole of the methylene group tilts with respect to the surface normal.^{30–32}

Further structural information comes from the fact that no spectral evidences for the CH and the NH stretch modes in the phenyl ring and ammonium group are found, which typically appear near 3075 and 3315 cm^{-1} , respectively.^{33–35} Considering the adjacent methylene group is well ordered, it is less likely that both phenyl and ammonium groups are randomly ordered. From theoretical consideration, we presume that the dipoles of the phenyl ring and the ammonium group tilt enough to yield no SFG signal, although we cannot determine the detailed orientation of these groups at present.^{30–32} The absence of SFG signal for the CH stretch in the chiral center is presumably due to the weak dipole.

In Figure 2, SFG peak intensity (depth) of both modes increases as the electrode potential is raised. Generally, SFG signal intensity is governed by the orientation and the number density of the adsorbed molecules according to eqs 1 and 2. One possible explanation for the enhanced SFG intensity is that the adsorbed Phe molecules are better ordered at higher electrode potential. To confirm this, we analyzed the SFG data in a quantitative fashion to obtain the oscillator strengths (A_{ν}) of the CH_2 stretch modes using eq 1. Figure 3 shows the ratio of oscillator strengths of the CH_2 (a) and the CH_2 (s) modes, $A_{\text{CH}_2(\text{a})}/A_{\text{CH}_2(\text{s})}$, as a function of external potential. It is found that the magnitude of the CH_2 (a) mode increases faster than that of

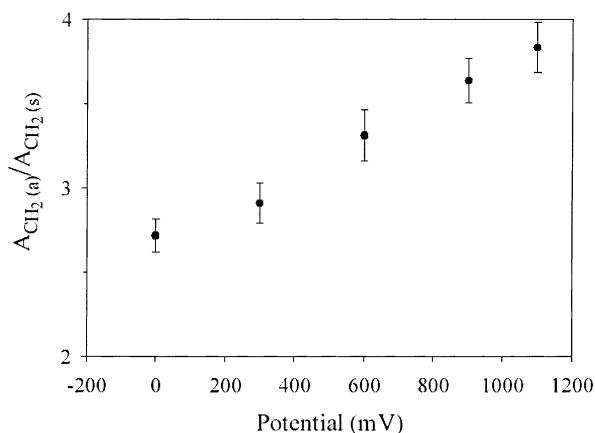


Figure 3. Ratio of CH_2 (a)/ CH_2 (s) vibrational mode strengths (A_s), $A_{CH_2(a)}/A_{CH_2(s)}$, from Figure 2 as a function of electrode potential.

CH_2 (s) as the potential is raised. The results suggest that the dipole component of the methylene group with respect to the surface normal is potential-dependent, probably due to the rearrangement of the adsorbed Phe molecules.^{31,32,36} Another possibility for the enhancement of SFG signal intensity is the adsorption of more Phe molecules due to the increased interaction between amino acids and electrode at higher potential.

On the bases of the SFG results above, we propose the adsorption behavior of Phe on GCE. At the positively charged surface, Phe molecules are adsorbed through the coordination of the carboxyl group to the GCE surface.³⁷ The methylene group adopts a conformation in which the dipole tilts with respect to the surface normal. As the potential becomes highly positive, the adsorbed Phe molecules undergo structural rearrangement giving rise to the dipole of the methylene group more tilted with respect to the surface normal. The phenyl ring and the ammonium groups are oriented in a way that the tilting angle from the surface normal is out of the range to produce observable SFG spectral evidences under the ssp polarization combination over the potential range investigated.

In summary, we have investigated the adsorption of Phe using SFG vibrational spectroscopy by applying external potential at the GCE/electrolyte interface. Experiments using partially deuterated Phe (Phe- d_2) revealed that the methylene group (CH_2) adjacent to the phenyl ring is responsible for the CH stretch modes in the SFG spectra. Changes in the ratio of the SFG signal intensity for the CH_2 (a) and CH_2 (s) are observed by varying potential indicating the reorientation of the adsorbed Phe. Currently, further structural investigations of Phe, other amino acids, peptides, and proteins at the electrode/liquid interfaces are underway in our laboratory.

Acknowledgment. This work was supported by the Director, Office of Science, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering, of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

Supporting Information Available: Details of the laser system and data collection procedures. Table S1 of curve fitting results for Figure 2; Figure S1 for the relative magnitude of nonresonant SFG signal from ITO, gold, GCE, and *atatic-*

polypropylene; and Figure S2 for SFG spectra of the partially deuterated Phe. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) Malmsten, M.; Veide, A. *J. Colloid Interface Sci.* **1996**, *178*, 160–167.
- (2) Burns, N. L.; Holmberg, K.; Brink, C. *J. Colloid Interface Sci.* **1996**, *178*, 116–122.
- (3) Roddick-Lanzilotta, A. D.; Connor, P. A.; McQuillan, A. J. *Langmuir* **1998**, *14*, 6479–6484.
- (4) Lori, J. A.; Hanawa, T. *Corros. Sci.* **2001**, *43*, 2111–2120.
- (5) Palit, D.; Moulik, S. P. *J. Colloid Interface Sci.* **2001**, *239*, 20–26.
- (6) Kubota, L. T.; Gambero, A.; Santos, A. S.; Granjeiro, J. M. *J. Colloid Interface Sci.* **1996**, *183*, 453–457.
- (7) Li, H.-Q.; Chen, A.; Roscoe, S. G.; Lipkowski, J. *J. Electroanal. Chem.* **2001**, *500*, 299–310.
- (8) Imamura, K.; Mimura, T.; Okamoto, M.; Sakiyama, T.; Nakanishi, K. *J. Colloid Interface Sci.* **2000**, *229*, 237–246.
- (9) Huerta, F.; Morallón, E.; Vázquez, J. L.; Aldaz, A. *J. Electroanal. Chem.* **1999**, *475*, 38–45.
- (10) Ogura, K.; Kobayashi, M.; Nakayama, M.; Miho, Y. *J. Electroanal. Chem.* **1998**, *449*, 101–109.
- (11) Stewart, S.; Fredericks, P. M. *Spectrochim. Acta A* **1999**, *55*, 1641–1660.
- (12) Kim, J.; Kim, G.; Cremer, P. S. *J. Am. Chem. Soc.* **2002**, *124*, 8751–8756.
- (13) Kim, J.; Cremer, P. S. *J. Am. Chem. Soc.* **2000**, *122*, 12371–12372.
- (14) Baldelli, S.; Markovic, N.; Shen, Y.-R.; Somorjai, G. A. *J. Phys. Chem. B* **1999**, *103*, 8920–8925.
- (15) Baldelli, S.; Mailhot, G.; Ross, P.; Somorjai, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 7697–7702.
- (16) Ong, T. H.; Davies, P. B.; Bain, C. D. *J. Phys. Chem.* **1993**, *97*, 12047–12050.
- (17) Bain, C. D.; Davies, P. B.; Ong, T. H.; Ward, R. N. *Langmuir* **1991**, *7*, 1563–1566.
- (18) Shen, Y. R. *Nature* **1989**, *337*, 519–525.
- (19) Shen, Y. R. *Surf. Sci.* **1994**, *299/300*, 551–562.
- (20) Gragson, D. E.; McCarty, B. M.; Richmond, G. L. *J. Am. Chem. Soc.* **1997**, *119*, 6144–6152.
- (21) Dekanski, A.; Stevanovic, J.; Stevanovic, R.; Nikovic, B. Z.; Jovanovic, V. M. *Carbon* **2001**, *39*, 1195–1205.
- (22) Bourdillon, C.; Demaille, C.; Moiroux, J.; Saveant, J. M. *J. Am. Chem. Soc.* **1995**, *117*, 11499–11506.
- (23) Kagan, M. R.; McCreery, R. L. *Langmuir* **1995**, *11*, 4041–4047.
- (24) Wang, Z.; Liu, D.; Dong, S. *Bioelectrochemistry* **2001**, *53*, 175–181.
- (25) Jenkins, G. M.; Kawamura, K. *Nature* **1971**, *231*, 175–176.
- (26) Lipkowski, J.; Shi, Z.; Chen, A.; Pettinger, B.; Bilger, C. *Electrochim. Acta* **1998**, *43*, 2875–2888.
- (27) Zhang, D.; Shen, Y. R.; Somorjai, G. A. *Chem. Phys. Lett.* **1997**, *281*, 394–400.
- (28) Gragson, D. E.; Richmond, G. L. *J. Phys. Chem. B* **1998**, *102*, 3847–3861.
- (29) Dawson, R. M. C.; Elliot, D. C.; Elliot, W. H.; Jones, K. M., Eds. *Data for Biochemical Research*, 3rd ed.; Clarendon Press: Oxford, 1986.
- (30) Wei, X.; Hong, S.-C.; Zhuang, X.; Goto, T.; Shen, Y. R. *Phys. Rev. E* **2000**, *62*, 5160–5172.
- (31) Akamatsu, N.; Domen, K.; Hirose, C. *J. Phys. Chem.* **1993**, *97*, 10070–10075.
- (32) Hirose, C.; Yamamoto, H.; Akamatsu, N.; Domen, K. *J. Phys. Chem.* **1993**, *97*, 10064–10069.
- (33) Gautam, K. S.; Schwab, A. D.; Dhinojwala, A. *Phys. Rev. Lett.* **2000**, *85*, 3854–3857.
- (34) Zhang, D.; Dougal, S. M.; Yeganeh, M. S. *Langmuir* **2000**, *16*, 4528–4532.
- (35) Simonelli, D.; Shultz, M. J. *J. Chem. Phys.* **2000**, *112*, 6804–6816.
- (36) Opdahl, A.; Phillips, R. A.; Somorjai, G. A. *J. Phys. Chem. B* **2002**, *106*, 5212–5220.
- (37) We could not observe SFG signal intensity for carbonyl group around 1700 cm^{-1} primarily due to the weak infrared intensity. This work is currently underway by the use of improved laser and OPG/OPA systems.