Discrimination among Amino Acids Using an Amperometric Biosensor Based on Electrochemical Nonlinearity

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(Received January 30, 1998; CL-980069)

We propose a novel sensing system based on the multi-dimensional information contained in a dynamic nonlinear response. A sinusoidal potential was applied to an amperometric L-amino acid sensor, and the resulting current of the sensor was analyzed by fast Fourier transformation (FFT). The amplitudes of the higher harmonics of the FFT characterize the nonlinear properties of the response. The amplitudes of the higher harmonics of the FFT exhibit characteristic changes which depend on the concentration and the chemical structure of amino acids at the sensor surface.

Amperometric biosensors, which are used in the medical, food manufacturing system, and scientific fields, have been developed to achieve high selectivity for specific molecules.[1-3] However, such biosensors are not extremely selective for specific molecules, since they also respond to interferants in many biological samples, which may be oxidized at the electrode surface.[1-3] In L-amino acid sensors, an L-amino acid oxidase responds to several L-amino acids.[4-7] Although several attempts have been made to overcome this problem (e.g. modification of the surface to decrease permeability to the interferants, the lowering the electrode potential by using an electron-transfer mediator, or utilization of highly selective enzyme),[1-3] it is difficult to sufficiently reduce interferants solely on the basis of the one-dimensional static information (d.c. voltage or d.c. current) obtained with a single detector. Thus, it may be useful to develop a strategy other than the chemical-sensing based on one-dimensional static information.

Recently, we described novel sensing systems with an electrode or a semiconductor gas sensor based on the multi-dimensional information contained in dynamic nonlinear responses.[8-10] In these systems, a sinusoidal input signal is applied to an experimental system and the resulting output signal is analyzed by FFT. The dynamic nonlinear response is quantitatively evaluated in terms of the amplitude of the higher harmonics of FFT. We found that the amplitude of the higher harmonics changes characteristically, depending on the chemical structure and concentration of the chemical species.[8-10]

In the present paper, we report that it is possible to quantitatively characterize among several L-amino acids based on the electrochemical nonlinearity. The concentrations of amino acids were determined by the higher harmonics of the output signals.

A sinusoidal potential (f=0.08Hz; $0.6+0.2\cos 2\pi ft$(V)) was generated by a potentiostat (NPGS-2501, Nikko Keisoku Corp., Japan) connected to a waveform generator (FG120, Yokogawa Elec. Corp., Japan), and applied to an amperometric sensor.[8] The input sinusoidal potential and the output current were stored in a computer, and then Fourier-transformed to the frequency domain. The amperometric sensor consisted of a Pt disk electrode (diameter: 1.5 mm) as the working electrode, a Ag/AgCl electrode as the reference electrode, and a Pt wire (diameter: 0.5 mm, length: 20 mm) as the counter electrode. A filter membrane (diameter: 5 mm, pore size: 5 μm, thickness: 0.16 mm) was immersed into a 0.1 M phosphate buffer solution (PBS, pH: 7.0, volume: 1 ml) with an 7 mg/ml L-amino acid oxidase for 1 hours. The filter membrane with the L-amino acid oxidase was attached to the Pt disk surface, and then covered with a semipermeable membrane (pore size 2.5 nm, thickness: 2.3 μm) to immobilize the membrane. All measurements were performed with stirring the test solution at 313±0.5K. The L-amino acid oxidase (EC 1.4.3.2, 0.36 units/mg) and other reagents were purchased from Sigma.

Figure 1 shows the output current vs. the input potential for (a) L-leucine, (b) L-isoleucine, (c) L-methionine, and (d) L-phenylalanine. The phase diagrams of current vs. potential for

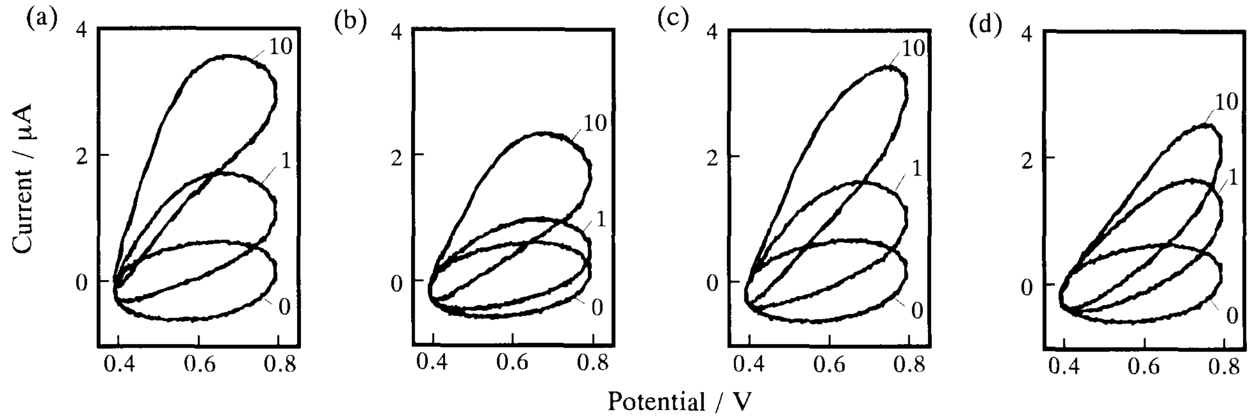

Figure 1. Current vs. potential curve for (a) L-leucine, (b) L-isoleucine, (c) L-methionine, and (d) L-phenylalanine. The rotational direction of the current vs. potential curve is clockwise. Numbers beside curves denote concentrations (mM) of L-amino acids.

individual amino acids have characteristic features. To qualitatively evaluate the electrochemical nonlinear responses, the relative amplitudes of the higher harmonics in FFT of the output current were examined (Figure 2). Here, R_n and I_n denote the real and imaginary part of the n-th harmonic in the FFT, and P_1 the power spectrum ($P_1=(R_1{}^2+I_1{}^2)^{1/2}$) of the fundamental harmonic (f=0.08 Hz)). In order to minimize the experimental error, the amplitudes of the higher harmonics are given as normalized values relative to P_1.[8,9] With the normalization procedure, the errors in the relative amplitudes were found to be less than 5% for all measurements in this study. The relative amplitudes change characteristically with an increase in the concentration. These results indicate that it is possible to determine the concentration of L-amino acid in the test solution on the basis of the information found in the higher harmonics of the sensor response. For example, the relative value of R_2 for phenylalanine little changed with an increase in concentration but that for other substances increased, therefore it is possible to distinguish among phenylalanine and other substances. As for the relative value of R_3, the response for leucine was enhanced although the dependencies for other substances was similar to one another. Although the responses for L-isoleucine was similar to those for L-methionine on the higher harmonics (Figure 2a,b,c), the difference in the responses between the two substances is characterized by the imaginary part of fundamental harmonic, I_1 (Figure 2d). Thus, it is possible to discriminate among L-amino acid using only a single electrode based on their dynamic electrochemical responses.

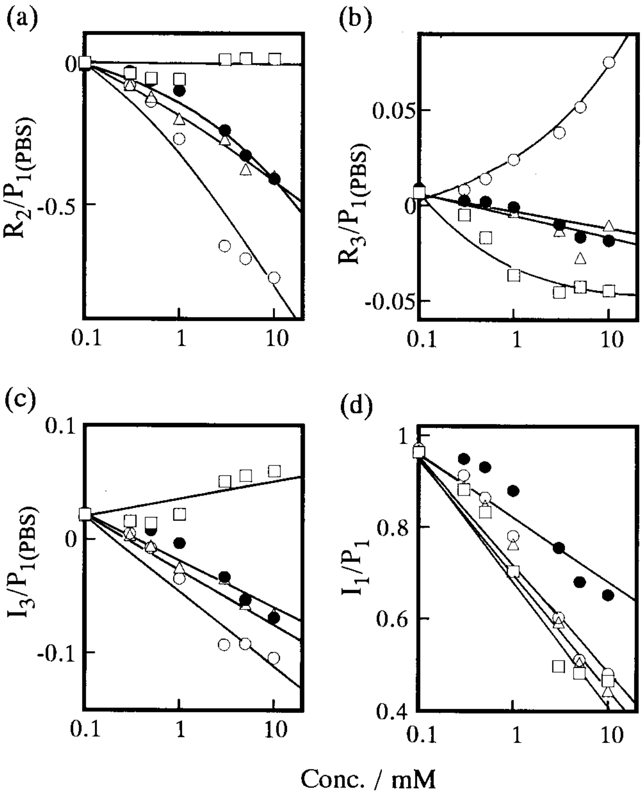

Figure 2. Relative amplitudes of higher harmonics in FFT of the output current for (○): L-leucine, (●): L-isoleucine, (△): L-methionine, and (□): L-phenylalanine. (a) $R_2/P_{1(PBS)}$, (b) $R_3/P_{1(PBS)}$, (c) $I_3/P_{1(PBS)}$, and (d) I_1/P_1. $P_{1(PBS)}$ denotes the power spectrum for the phosphate buffer solution alone.

These different nonlinear responses may be due to the kinetics in the electrochemical reaction.[8-10] The electrochemical reaction rates of L-amino acids on the electrode surface with L-amino acid oxidase, and the hysteresis in the current vs. potential curve, which is due to difference in the concentration of amino acid at the same potential, correspond to the real part and imaginary part of FFT, respectively. The degree of the hysteresis corresponds to the difference between the rate of the reaction of the L-amino acids on the electrode surface and the rate at which the electrical potential is changed. If the reaction rate of the amino acid is significantly higher than its diffusion rate at the scanning of potential, the degree of hysteresis changes depending on the rate at which the electrical potential is changed. The physicochemical significance of higher harmonics has been reported in the separate papers.[8-10] Thus, the differences in the responses among individual substances are enhanced by the application of the sinusoidal potential rather than d.c. potential.

Although our results and experimental system are only preliminary, it is apparent that the present method provides us abundant information based on the nonlinear dynamics of the reaction kinetics of L-amino acids on the electrode. To enhance the information content of the response of sensor, the change in the scanning rate, the coupling of different kinds of sensors, evaluation of the saturation effect is also useful for the measurement with the periodic change in the potential. With this system based on the dynamic nonlinearity, it will be possible to distinguish between chemical species in a mixture.[9] In addition, the characteristic responses for these substances should be physicochemically evaluated by considering the mass transfer and the kinetics of each substance on the electrode surface.

The present study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, the Shimadzu Science Foundation, and the Nestlé Science Promotion Committee.

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