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Detection and Characterization of Biological Compounds in Human Sweat Adsorbed on Alumina by Inelastic Electron Tunneling Spectroscopy

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Vibrational spectra of compounds in sweat adsorbed on alumina have been measured by inelastic electron tunneling spectroscopy. Analysis of the tunneling spectra showed that lactic acid and fatty acids are selectively detected from separated aqueous and benzene solutions, respectively.

Inelastic electron tunneling spectroscopy (IETS) reveals the vibrational spectrum of adsorbed species on the oxide surface of a metal/oxide/metal tunneling junction.[1] The high sensitivity and resolution give the detailed vibrational spectrum of a fraction of a monolayer of adsorbed species on the oxide surface. Tunneling spectra of various compounds, for example, carboxylic acids and aldehydes[1,2] have been measured and indicated their molecular structures on the oxide surfaces. These compounds were found to be adsorbed onto the alumina surfaces as carboxylate anions. Since tunneling spectroscopy has the great advantage for the detection of a very small quantity of samples adsorbed on the oxide surfaces, it can be a suitable analytical technique for biological compounds. Tunneling spectra of some amino acids, nucleosides, nucleotides, and nucleic acids adsorbed on alumina surfaces have been measured to show the usefulness of this technique in biological research.[1] However, no previous attempt has been made to apply tunneling spectroscopy to the study of biological compounds in body fluids. In the present letter, we report the first attempt to detect and characterize biological compounds in human sweat using tunneling spectroscopy. The tunneling spectra of the adsorbed species doped from aqueous and organic solutions have been obtained and analyzed in order to clarify which sweat components are important in adsorption on the alumina surfaces of the tunneling junctions.

Sweat was collected from the clean forehead of a healthy student (male, 22 years old) after running. Care was taken to assure the absence of toilet goods or cosmetics. The sweat (about 60 mg) and equal volume (3 mL) of distilled water and benzene were mixed and shaken for about 10 min at about 20°C in order to distribute biological compounds in the sweat to the aqueous and benzene phases. The mixture was left for 5 - 60 min and the phases were separated by centrifugation (3000 rpm, 5 min).

The junction preparation and apparatus for measuring the tunneling spectrum have been described previously.[3,4] Aluminum was evaporated from a molybdenum boat on a clean glass slide to form three strips (1 mm wide) at a pressure of 10^{-5} Torr (1 Torr = 133.322 Pa). The surfaces of the strips were oxidized in an oxygen dc glow discharge. One drop (about 60 μL) of the aqueous or benzene sweat solution was dropped on the strips and excess solution was immediately spun off. The junctions were completed with an evaporated Pb cross strip (1 mm wide).

The tunneling spectrum of the separated aqueous sweat solution doped onto the alumina surface of the tunneling junction is shown in Fig. 1. The spectrum has the strong characteristic peaks due to the stretching modes (ν) of CH at 2960 and 2910 cm^{-1}. The peaks caused by the deformational modes (δ) of CH are present at 1440 and 1360 cm^{-1}. The tunneling spectrum of the separated aqueous sweat solution suggests that a relatively simple organic compound is adsorbed on the alumina surface. The tunneling spectrum of lactic acid, $CH_3CH(OH)COOH$, the major organic component of sweat,[5,6] is also shown in Fig. 1. A detailed analysis and comparison of the tunneling spectrum of the sweat showed good agreement with that of lactic acid. The assignments of the tunneling spectra of the sweat and lactic acid were made with reference to those of the infrared spectra of lactic acid and its salts.[7] The tunneling spectrum of lactic acid has the peak due to the asymmetric stretching mode of the carboxylate group

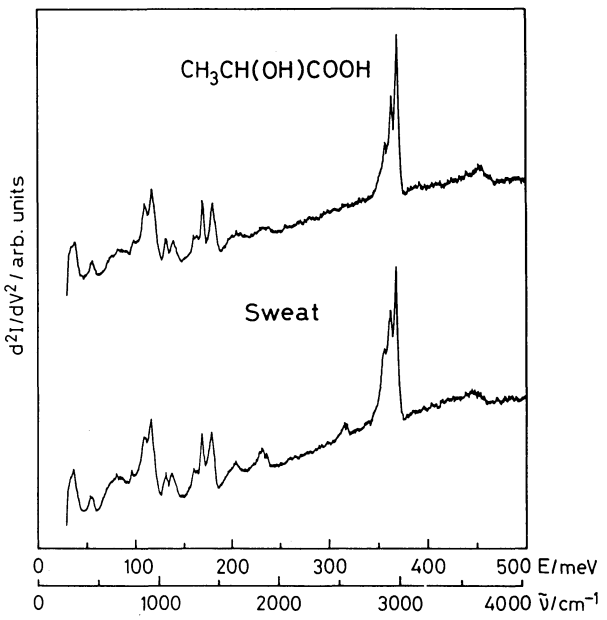

Fig. 1. Tunneling spectra of aqueous phase of sweat and lactic acid on Al_2O_3. The sweat (68.9 mg) was extracted with 3 mL of water and benzene. Lactic acid was doped from an aqueous solution (1.1 mg/mL).

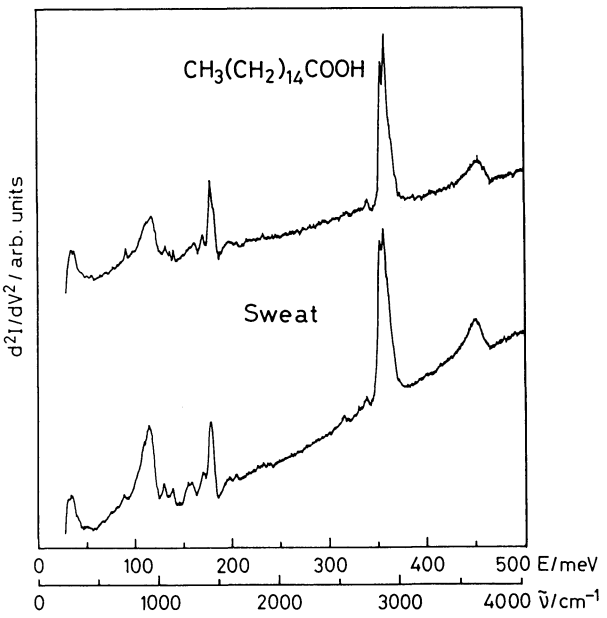

Fig. 2. Tunneling spectra of benzene phase of sweat and palmitic acid on Al_2O_3. The sweat (63.4 mg) was extracted with 3 mL of water and benzene. Palmitic acid was doped from a benzene solution (0.1 mg/mL).

($\nu_{as}COO^-$) at 1660 cm^{-1} and no $\nu C=O$ peak, showing that it is adsorbed as lactate anion on the alumina surface. The tunneling spectrum of the sweat also has the $\nu_{as}COO^-$ peak at 1650 cm^{-1}. This finding is consistent with the fact that many carboxylic acids are adsorbed on the metal-oxide surfaces as carboxylate anions.[1,2] The tunneling spectrum of urea, the second major organic component of sweat,[5,6] doped from its aqueous solution (10 mg/mL) was measured. However, it disagreed with the tunneling spectrum of the sweat. The tunneling spectra of other organic components of sweat, for example, phenol, pyruvic acid, uric acid, and creatinine were measured by use of their aqueous solutions (1 mg/mL) and also disagreed. Thus, we conclude that lactic acid in the sweat is distributed to the aqueous phase and selectively adsorbed onto the alumina surfaces.

Lactic acid is the major organic component of sweat secreted by human eccrine glands and is derived from blood glucose. Its concentration depends on such factors as sweat rate, heat exposure, exercise, and acclimatization.[5,6] Analytical studies on lactic acid in sweat have been done by fluorescence microscopy[8] and liquid ionization mass spectrometry.[9] In the present study, the combination of tunneling spectroscopy and solvent extraction showed that lactic acid in sweat is easily and selectively detected from the aqueous solution. It is concluded that lactic acid reacts with the surface OH groups on alumina to give adsorbed lactate anion. Because the tunneling junctions are prepared in high vacuum and the lactic acid is adsorbed as the carboxylate anion on the surfaces, the adsorbed lactate is considered to be present of the order of a monolayer on the alumina surfaces of the tunneling junctions. The tunneling spectra of lactic acid doped from the concentration in the range 0.1 - 1.1 mg/mL showed no spectral change, suggesting the saturated monolayer coverage of the surfaces.

The tunneling spectrum of the separated benzene sweat solution is shown in Fig. 2. The spectrum has the strong characteristic peaks due to νCH of the adsorbed species at 2860 and 2830 cm^{-1}. The peaks caused by δCH are present in the frequency range 1250 - 1440 cm^{-1}. The peak of νCC is present at 1050 cm^{-1}. The tunneling spectrum of the separated benzene sweat solution suggests that organic compounds having long alkyl chains are adsorbed on the alumina surface. We measured tunneling spectra of many organic compounds and found that fatty acids with long alkyl chains ($CH_3(CH_2)_{n-2}COOH$, n=10-18) give similar tunneling spectra to that of the benzene sweat solution. The tunneling spectrum of palmitic acid (n=16) is shown for example in Fig. 2. The assignments of the tunneling spectrum of the sweat were made with reference to those of palmitate anion on alumina.[2] The tunneling spectrum of palmitic acid has the weak and broad peak due to $\nu_{as}COO^-$ at 1590 cm^{-1} and no $\nu C=O$ peak, showing that it is adsorbed as palmitate anion on the alumina surface. The tunneling spectrum of the sweat has also the $\nu_{as}COO^-$ peak at 1590 cm^{-1} and no $\nu C=O$ peak. Thus, we conclude that fatty acids in the sweat are distributed to the

benzene phase and selectively adsorbed onto the alumina surfaces. However, neither fatty acids are involved in the organic components of sweat[5,6] nor have they been detected in the analytical studies.[8,9]

Skin lipid secreted by sebaceous glands comprises triglycerides, wax esters, squalene, and free fatty acids.[10,11] The major fatty acids found in the lipids have chain lengths from 14 to 18 carbon atoms. Kosugi and Ueta[10] have reported the separation and detection of fatty acids in the lipids from faces of several young Japanese men using chromatography. Though a relatively large quantity of branched chain and odd-carbon-number fatty acids have been found, the major component is palmitic acid. These results are consistent with those of the present study.

We have reported for the first time that IETS is applicable to the detection and characterization of the biological compounds in human sweat. Only one drop of sweat ($\sim$ 60 mg) is sufficient and no complex pretreatment is necessary. The combination of solvent extraction and tunneling spectroscopy enables us to analyze rapidly and selectively the biological compounds in sweat. Lactic acid is distributed to the aqueous phase and selectively adsorbed onto the alumina surfaces as lactate anion. Fatty acids with long alkyl chains are distributed to the benzene phase and adsorbed as carboxylate anions. The fatty acids are considered to be collected with the sweat. These adsorbed species are present of the order of a monolayer on the surfaces. Further investigations on biological compounds in sweat adsorbed on alumina are now in progress.

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