Specific Adsorption of Bitter Substances on Lipid Bilayer-coated Piezoelectric Crystals

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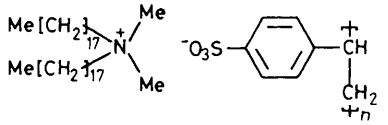
The adsorption of various bitter substances on a lipid bilayer matrix was detected by observing the frequency change of a synthetic bilayer-coated piezoelectric crystal; there was a good correlation between the partition coefficient of the bitter material (between the aqueous phase and the synthetic lipid bilayer film on the crystal) and the bitter taste threshold concentration in the biological cell.

The use of the piezoelectric crystal as a microbalance is well known: the frequency of the crystal decreases linearly with the amount of substances adsorbed.[1] Synthetic polymer- or natural protein-coated piezoelectric crystals have been studied as selective detectors for various toxic vapours such as NH_3, SO_2, and parathion in the gas phase.[2,3]

Recently, it has been suggested that bitter substances may be detected by direct adsorption on a lipid bilayer matrix without a specific receptor in gustatory cells, although sweet substances and amino acids are recognized by specific receptor protein molecules in the membranes of taste cells.[4] This finding prompted us to prepare a piezoelectric crystal coated with a simple lipid bilayer marix without protein; this crystal can detect selectively various bitter substances in aqueous solution.



$2C_{18}N^+2C_1/PSS^-$

As a simple lipid bilayer matrix, a synthetic multibilayer-immobilized film $2C_{18}N^+$ $2C_1/PSS^-$ was used, prepared as a polyion complex from dioctadecyldimethylammonium bromide $2C_{18}N^+2C_1Br^-$ and sodium poly(styrenesulphonate) (PSS^- Na^+).[5] The $2C_{18}N^+$ $2C_1/PSS^-$ bilayer film was cast from chloroform solution on both sides of a silver-electrode-deposited piezoelectric crystal (9 MHz; AT-cut). The cast film was transparent, water-insoluble, and estimated to be 0.5 ± 0.1 μm thick from a scanning electron microscopic observation. *X*-Ray diffraction analyses showed that $2C_{18}N^+2C_1$ amphiphiles form extended lamellar structures of lipid bilayers (38 Å thick) parallel to the film plane in the polyion complex with PSS^-. The bilayer film on the crystal showed a sharp endothermic peak at 45 °C in differential scanning calorimetry (DSC) in aqueous solution, corresponding to the phase transition from solid to fluid liquid crystalline state.[5] The quartz crystal (9 MHz) was driven at 5 V d.c. and the frequency of the vibrating crystal was measured in distilled water by use of an Iwatsu frequency counter attached to a microcomputer system (NEC, model PC 8801). Bitter substances were injected as ethanolic solutions (5—50 μl) into distilled water (10 ml) with stirring.

Figure 1 shows the frequency changes of the bilayer-coated piezoelectric crystal in water at 45 °C responding to the addition of strychnine, a typical bitter substance. The frequency of the crystal immediately decreased (ΔF 507 ± 10 Hz) within 30 s after the addition of strychnine (19.3 p.p.m.), and reverted to the original value on immersing the crystal in distilled water. Thus, the adsorption of the bitter substance is reversible in aqueous solution. When an uncoated crystal or a crystal coated with bovine plasma albumin (natural proteins), synthetic poly(vinyl alcohol), or polystyrene was employed, the response of the frequency to bitter substances (strychnine, quinine, papaverine, picric acid, and octa-acetyl sucrose) was negligible small ($\Delta F < 10$ Hz). The bilayer-coated crystal hardly responded to a sweet substance (sugar) or to L-glutamic acid. Thus bitter substances are specifically adsorbed on the lipid bilayer on the piezoelectric crystal, and decrease the frequency. The amount of adsorbed bitter substance can be calculated from equation (i),[1] where ΔF is the frequency change (Hz), F is the basic frequency of the crystal (9 MHz), Δm is the amount of substance adsorbed (g), and A is the area of the electrode of the crystal (0.196 cm^2).

$$-\Delta F = (2.3 \times 10^6)F^2(\Delta m/A) \quad \text{(i)}$$

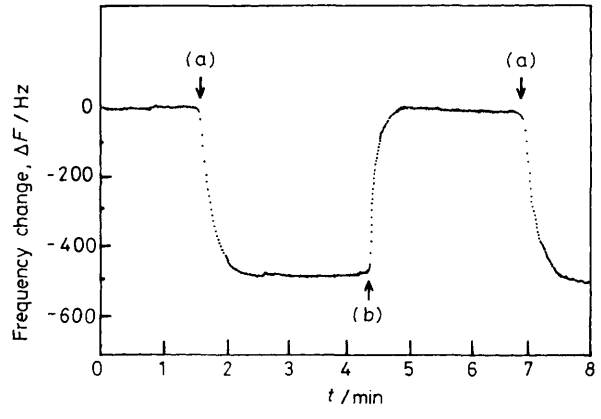


Figure 1. Typical piezoelectric response of the bilayer-coated crystal to the addition of strychnine in aqueous solution at 45 °C. An ethanolic solution (50 μl) of strychnine was injected into distilled water (10 ml) at the arrow (a), giving 19.3 p.p.m. of strychnine; the aqueous solution was changed to distilled water at the arrow (b).

Figure 2 shows the temperature dependence of the frequency change and the amount of strychnine adsorbed on the piezoelectric crystal upon addition of 19.3 p.p.m. of strychnine in water. In the case of uncoated and polystyrene-coated crystals, strychnine was scarcely adsorbed on crystals, regardless of the temperature employed. However, adsorption on the $2C_{18}N^+2C_1/PSS^-$ bilayer-coated crystal was large and specifically increased near 40—45 °C, close to the phase

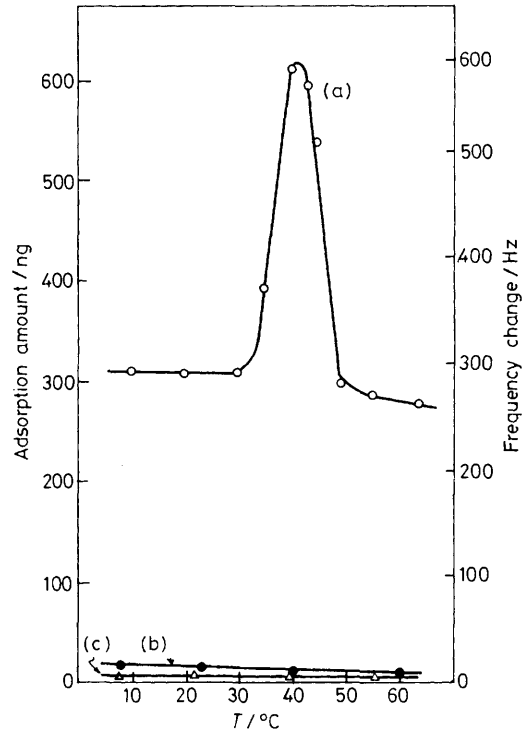


Figure 2. Temperature dependence of adsorption on piezoelectric crystals upon addition of 19.3 p.p.m. of strychnine. (a) $2C_{18}N^+$-$2C_1/PSS^-$ bilayer-coated crystal, (b) polystyrene-coated crystal, (c) uncoated crystal.

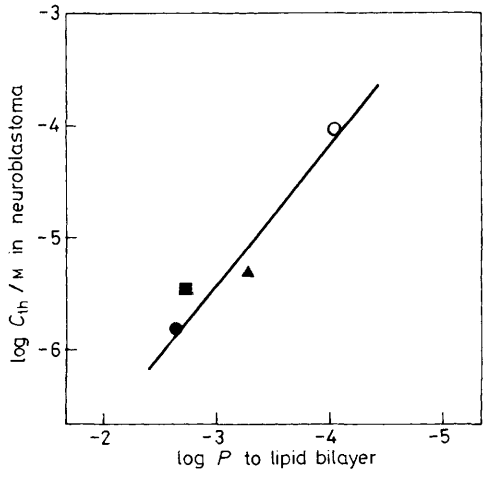


Figure 3. Relation between threshold concentration (C_{th}) of bitter substance in the neuroblastoma cell (ref. 6) and their partition coefficients (P) between the bilayer-coated crystal and aqueous solution at 45 °C: ●, strychnine; ■, quinine; ▲, papaverine; ○, octa-acetylsucrose.

transition temperature (T_c 45 °C) for the lipid bilayer on the crystal obtained from DSC measurements. Similar temperature dependence was observed on addition of various bitter substances other than strychnine. Thus substances are specifically adsorbed near T_c, where solids coexist with fluid liquid crystals in the lipid bilayers.

Adsorption experiments were carried out on four typical bitter substances (octa-acetylsucrose, quinine, papaverine, and strychnine) over a wide range of concentrations (0.1—100 p.p.m.) at 45 °C. The amounts adsorbed increased linearly with increase in concentration of the bitter substance in water, and the partition coefficients (P) of the bitter substances between the lipid bilayers and the aqueus phase were calculated from the slope. Figure 3 shows a plot of log P against the logarithm of the minimum concentration of the bitter substance (threshold value, C_{th}) for induced depolarization of the mouse neuroblastoma cell (N-18 clone). The neuroblastoma cell was used by Kurihara and his co-workers as a model of a bitter taste cell, the membrane potential of which is reversibly depolarized by the addition of bitter substances.[6] There was a good correlation between the threshold values C_{th} and the partition coefficients of the four bitter substances, the bitter materials having the lower threshold values showing higher partition coefficients towards the synthetic bilayer. The log P value also showed a good correlation with the logarithm of the bitter taste threshold concentration in man.[6,7]

In conclusion, the synthetic bilayer-coated piezoelectric crystal acts as a simple sensor system for bitter substances in aqueous media; this suggests that bitter taste is induced by adsorption on the lipid bilayer matrix of the gustatory receptor membrane in biological taste cells.

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