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# Amino Acid and Urea Sensors Using Parsley Leaves as Catalytic Material

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The minced parsley leaves coupled with potentiometric ammonia gas sensing electrode showed effective biocatalytic activity, i.e. responded for L-glutamine, L-asparagine and urea with a Nernstian slope of 47-48 mV per decade of substrate concentrations. Very good time stability with no significant loss of biocatalytic activity over 40 days was maintained.

Plant substances have been used as biocatalytic layers coupled with potentiometric or amperometric techniques.[1] The specialized structures of plants, e.g. leaves, [2] fruit, [3] flower, [4,5] etc., offer particularly attractive properties as biocatalysts because structures related to growth, reproduction, and nutrient storage concentrate and stabilize highly selective biocatalytic activity. After carrying out the preliminary screening test for some sorts of plant, we found the minced parsley (Petroselinum crispum) leaves coupled with potentiometric ammonia gas sensing electrode show effective biocatalytic activity, i.e. selective deamination responses for certain amino acids and urea, and can be usable as biosensor for these substances. Its principle is thought to be the same as that of usual enzyme immobilized sensors. A substrate such as L-glutamine in the solution passes through the membrane, diffuses into plant tissue and decomposes to ammonia and other substances by the catalytic action of the enzyme contained in parsley. Then, produced ammonia responds to the ammonia electrode.

An Orion Model 95-10 ammonia gas electrode was used in the construction of the biosensors. Potentiometric measurements were carried out by using a Corning Model SA 720 ion meter. Parsley used was commercially available one (growing district,

Chiba Prefecture, Sanbu-gun) and supplied if necessary. Its catalytic activity did not change through the experimental period from July to next January. Parsley leaves were minced within 1 x 1 mm small pieces by a razor blade or ground in a agate mortar with a pestle. By using such a minced leaves, the activity and a response time are almost the same and only depend on the amount of immobilized tissue (see later). Minced leaves of 1-10 mg parsley were attached to the surface of ammonia electrode covered by dialysis membrane and set by O-ring. The parsley immobilized electrode was dipped in 0.1 mol $dm^{-3}$ Tris-HCl buffer solution. To find out the response on this electrode, various sorts of amino acid or urea solution were added to buffer solution and the potential change due to $NH_3$ liberated from the substrate by the catalytic action of the enzyme contained in parsley was followed.

Figure 1 shows a typical example of calibration curve with a slope of 48 mV per decade for L-glutamine concentrations in the 3 X $10^{-4}$ to 3 X $10^{-3}$ mol $dm^{-3}$ range with a detection limit of 1 X $10^{-5}$ mol $dm^{-3}$. Increasing the amount of

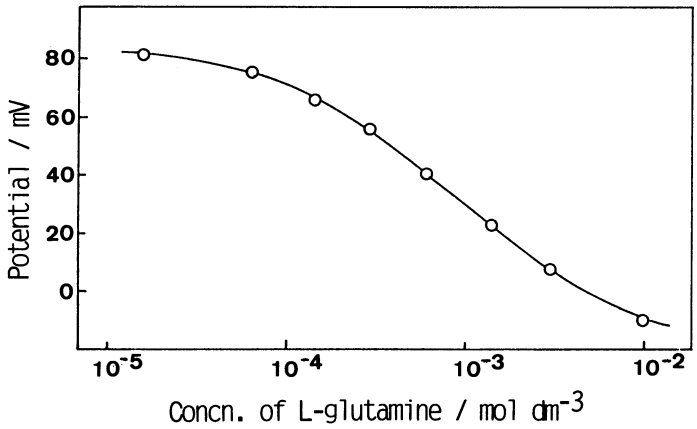


Fig.1. Calibration curve for L-glutamine using 5 mg immobilized minced parsley leaf electrode in 0.1 M Tris-HCl buffer at pH 7.4, 30 °C.

immobilized tissue from 1 mg to 10 mg produces a modest improvement in response slope, but at the expense of response time as shown in Fig. 2. The pH dependency on response slope was measured from pH 7.0 to 8.8 by using 2 mg of parsley leaves at 30 °C. Within these pH range, the response slope did not change so much(Fig. 3). The activity of this electrode immediately after the immobilization of minced tissue was not so high, but it showed the highest value after a few days, and was kept for more than forty days. This parsley immobilized electrode also showed a

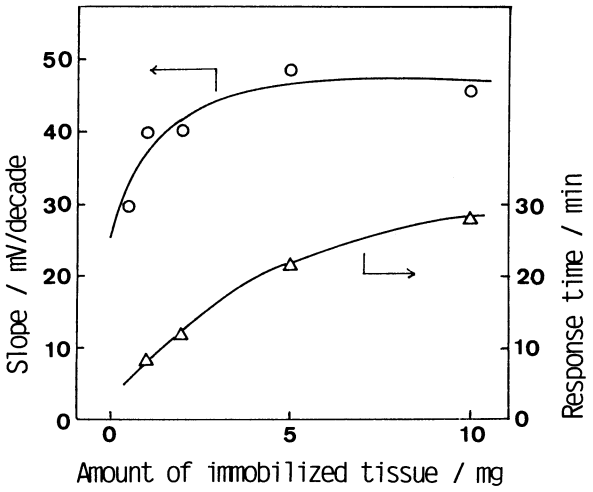


Fig.2. Relationship between amount of immobilized parsley leaf and slope( O ) or response time( △ ) at pH 7.4, 30 °C.

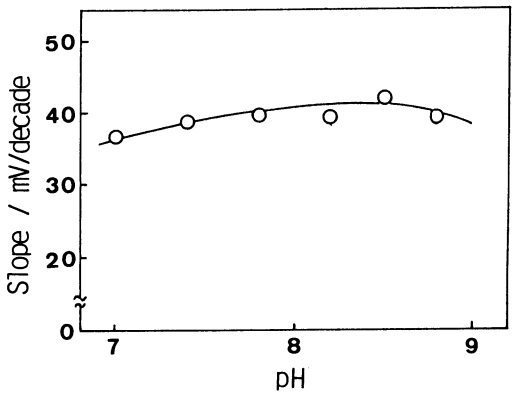


Fig.3. Relationship between pH and slope of immobilized parsley electrode for L-glutamine using 2 mg minced leaf at 30 °C.

Table 1 Selectivity of parsley electrode immobilized minced leaf in 0.1M Tris-HCl buffer at pH 7.4, 30 °C

| Substrate | Amount of tissue(mg) | Response (mV/decade) | Substrate | Amount of tissue(mg) | Response (mV/decade) |
|---|---|---|---|---|---|
| $NH_4Cl$ | 2 | 58 | L-phenylalanine | 2 | 2 |
| L-glutamine | 5 | 48 | L-ornitine | 2 | 3 |
| D-glutamine | 1 | 38 | L-leucine | 2 | 2 |
| L-asparagine | 5 | 48 | L-citrulline | 2 | 1 |
| D-asparagine | 1 | 26 | L-glycine | 2 | 0 |
| L-arginine | 2 | 10 | L-methionine | 2 | 0 |
| L-serine | 2 | 13 | L-lysine | 2 | 0 |
| L-threonine | 2 | 10 | L-tryptphan | 2 | 0 |
| L-alanine | 2 | 6 | Formamide | 2 | 0 |
| L-glutamic acid | 2 | 5 | Urea | 10 | 47 |
| L-aspartic acid | 2 | 5 | Thiourea | 2 | 0 |
| L-histidine | 2 | 5 | $NaNO_2$ | 2 | 0 |
| L-valine | 2 | 4 | | | |

good response at pH 7.4 by using 5 mg tissue for L-asparagine with a slope of 48 mV per decade in the $2 \times 10^{-4}$ to $2 \times 10^{-3}$ mol $dm^{-3}$ and for urea with a slope of 47 mV per decade in the $4 \times 10^{-4}$ to $2 \times 10^{-4}$ mol $dm^{-3}$ concentrations range. This electrode also responded to D-glutamine and D-asparagine with a lesser slope of 38 mV and 26 mV respectively (Table 1). Such a response pattern suggests that the primary biocatalytic activity involves the enzyme glutamine-(asparagine-)ase,

E.C.3.5.1.38.[5] A principal feature of this enzyme is that it catalyzes the hydrolysis of the D-isomers of glutamine and asparagine with a less activity than L-isomers, which is in accord with our case. The experimental data also suggests parsley leaves contain urease. For other amino acids and nitrogen containing substances, parsley immobilized electrode responded with a slope of under 10 mV or did not respond. These are tabulated in Table 1.

Though the selectivity of this parsley immobilized electrode is not so good, it is usable as a biosensor for L-glutamine, L-asparagine and urea respectively. This simple technique also offers a very useful method for enzyme screening in plant substances.

References

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