Discrimination between D- and L-Tryptophan Amino Acids Using Resistance Change of a Lipid Membrane

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Discrimination between D- and L-Tryptophan Amino Acids Using Resistance Change of a Lipid Membrane

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Abstract: D-tryptophan and L-tryptophan are two amino acids which are optical isomers. It was impossible to differentiate L-tryptophan from D-tryptophan in a previous report using a measuring technique of the membrane potential of a multichannel taste sensor with lipid membranes. In this work, the two amino acids were identified from each other using the resistance change of the lipid membranes by performing the impedance measurement.

Keywords: Amino acids, Optical isomer, Impedance measurement, Lipid membrane

1. Introduction

As highlighted in earlier studies,[1-4] a multichannel taste sensor using lipid membranes is a powerful device to discriminate and quantify the taste of foodstuffs but is not adequate for measuring weak electrolytes and non-electrolytes as it is for strong electrolytes. This is because the response electric potential in lipid membranes of the taste sensor is attributed to the change in the surface potential produced by the diffuse electric double layer whereas non-electrolytes and weak electrolytes insignificantly affect it.[5]

Detection of taste substances using impedance change of the phospholipid Langmuir-Blodgett lipid membrane[6,7] dioctyl phosphate (DOP), oleyl amine (OAm) and trioctyl methyl ammonium chloride (TOMA) lipid membranes[9] was successfully reported in previous papers. There is therefore a feasibility of this technique for the cases where the results of the conventional taste sensor of potentiometric type are not very good.

Two kinds of amino acids, D-tryptophan and L-tryptophan, are two optical isomers. L-tryptophan amino acid is known to be bitter while D-tryptophan tastes sweet. In **Fig. 1**, the two optical isomers are shown. Development of technique to discriminate the isomers has been strongly required in fields of chemical industry, medical science and pharmaceutics. These two kinds of amino acids could not be discriminated using the conventional taste sensor with lipid membranes.[8] Thus, the purpose of the present paper is to distinguish L- and D- tryptophans using impedance measurement of lipid membrane.

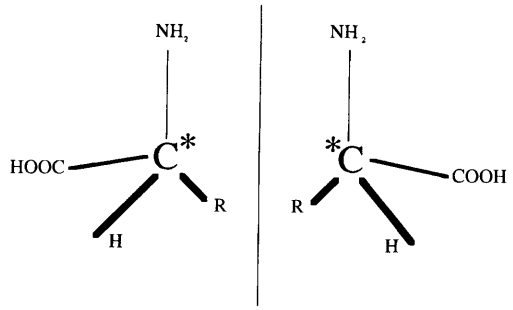

Fig.1 Two optical isomers, L-tryptophan and D-tryptophan amino acids.

2. Materials and Methods

2.1 Membrane

The lipid membrane used was made of poly vinyl chloride (PVC) as the forming polymer, the lipid was Span 20, which is a kind of sorbitan fatty acid ester, and di-n-octylphenylphosphonate (DOPP) as the plasticiser. 10μl of the membrane solution thus made was dropped on the electrode where it was allowed to dry for forming the membrane. **Figure 2** shows the electrode where the membrane is pasted.

2.2 Measuring Apparatus

Figure 3 shows the apparatus setup for the measurement of the lipid membrane impedance. Input current was generated by the oscillator and the voltage/current converter. The input current was applied to the membrane using a platinum counter electrode.

The voltage across the monitor resistance R_m,

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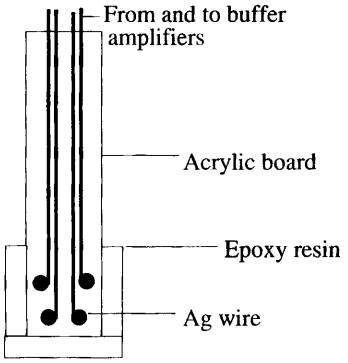

Fig.2 The electrode where the lipid membrane is pasted by drying.

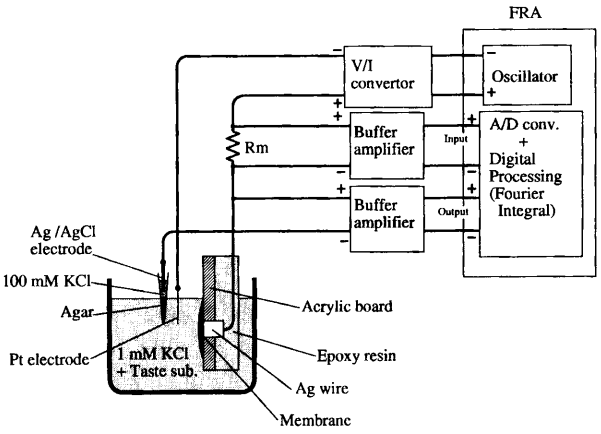

Fig.3 Setup for the measurement of the impedance of lipid membranes.

inserted in the input circuit, was measured and it gives the input current. Also, the voltage across the lipid membrane was measured by an Ag/AgCl electrode covered with an agar salt bridge. The A/D converter with digital processing was used for measuring the amplitudes and phases of both the input currents and the output voltages.

The amino acids, L- and D-tryptophan, were separately dissolved in a solution of 1 mM of KCl. The concentrations of the amino acids were increased in steps, and the input currents and the output voltages were measured at each step.

3. Membrane Resistance and Capacitance

The impedance of the membrane was calculated by the ratio of the amplitudes and the difference of phases between the input current and the output voltage. As explained in section 4.1, the membrane was considered as a parallel connection of resistance and capacitance. From a series of calculations, the resistance r and capacitance C of the lipid membrane were obtained for the angular frequency ω as follows:

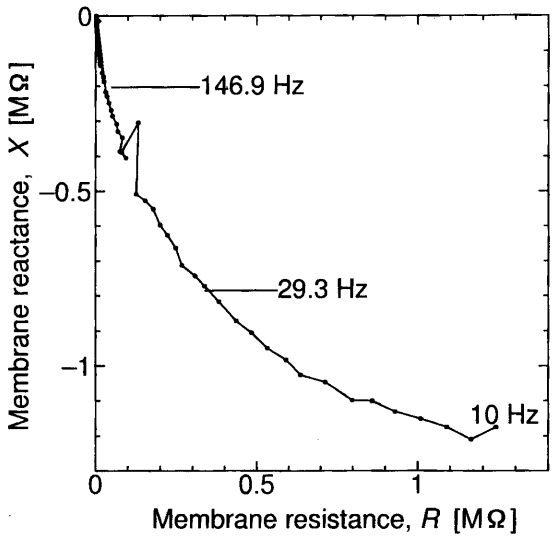

Fig.4 Complex plane showing the relationship between the reactance and the resistance of the lipid membrane.

$$r = R[1 + \frac{X^2}{R^2}], \tag{1}$$

$$C = -\frac{X}{\omega(R^2 + X^2)}, \tag{2}$$

where X and R are the imaginary and real part of the membrane impedance, respectively.

4. Results

4.1 Equivalent circuit of the Membrane

Figure 4 shows a complex plane showing the plot of the membrane reactance against the resistance. The trace shows a part of circle in the X-R plane. It implies that the equivalent circuit for the membrane impedance can be represented by a parallel connected circuit of resistance and capacitance. From this point of view, a series of caculations were done to find the resistance and capacitance of the membrane.

4.2 Impedance Changes Due to L- and D-Tryptophan

For each concentration of the amino acid, the resistances and capacitances were calculated. The differences between these resistances and the initial resistances (resistance when there was no any amino acid in the KCl solution) were calculated and plotted against amino acid concentrations as shown in **Fig. 5**.

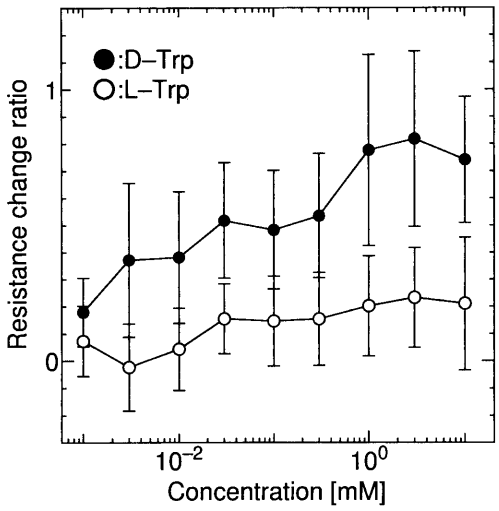

Fig.5 Change of the lipid membrane resistance due to increasing concentration of L- and D-tryptophan amino acids. Membranes with the same resistance range at 10 Hz and 0 mM of either amino acid were considered.

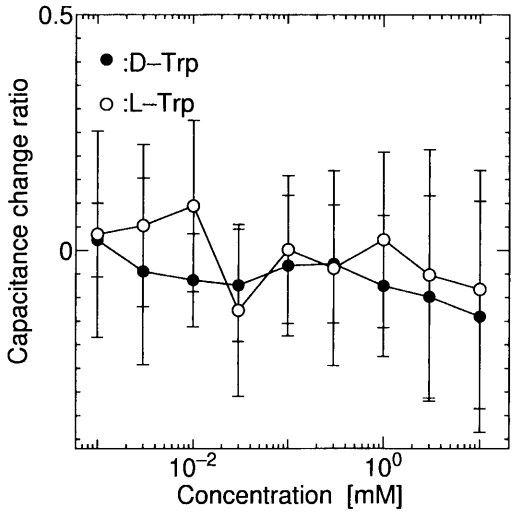

Fig.6 Change of the lipid membrane capacitance due to increasing concentration of L- and D-tryptophan amino acids.

Membranes with the initial resistances in the same range, 1-6 MΩ, were used in this comparison. The initial membrane resistances of 32 membranes studied here are shown in **Fig. 7**.

As shown in **Fig. 5**, the quantitative reproducibility is not so good. However, it can be seen that the resistance tends to increase at a higher rate for D-tryptophan amino acid than for L-tryptophan amino acid. This result shows that the two amino acids are successfully discriminated. We can therefore use this measuring method as a way of distinguishing between the two amino acids with different chirality. From the membrane capacitance, however, no distinctions were observable as shown in **Fig. 6**.

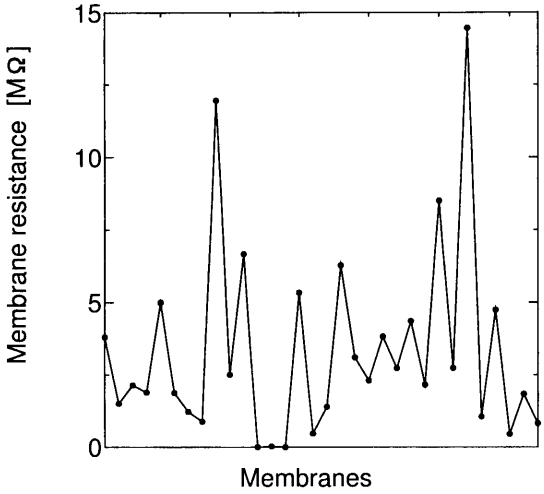

Fig.7 Membrane resistances at 10 Hz, 0 mM of either amino acid.

5. Discussion

As reported previously,[6,7] control of the membrane resistance was very difficult and it appears that it affected the results. The membrane resistances at the lowest frequency of 10 Hz were very different from one membrane to the others, as can be highlighted by **Fig. 7**. Comparisons for the increase in the lipid membrane resistance and capacitance with increasing L- and D-tryptophan amino acids in **Fig. 5** and **Fig. 6** were done at the frequency of 10 Hz. The reason why the frequency 10 Hz was chosen is that the membrane reactance and resistance had almost the same values, as can be understood from **Fig. 4**. In that case, the resistance r and capacitance C of the parallel circuit can be obtained at the most reliable accuracy.

In the previous work,[6] bitter substances tended to increase the impedance of the phospholipid Langmuir-Blodgett membrane. If we compare the two amino acids, D-tryptophan is sweet and L-typtophan is bitter; hence we expected that resistance would have increased at higher rate when we increase the L-tryptophan concentration. However, the result in **Fig. 5** was opposite to the expectation. Some of the bitter substances in the covered work[6] had alkaline earth metal ions which played a part in the increase of the LB membrane impedance. Also bitter substances such as quinine, strychnine or nicotine are strongly hydrophobic and enter the

alkyl-chain layer of the LB membrane. Although saccharose and sodium L-glutamate taste sweet and umami, respectively, they also increased the membrane resistance; hence, it was also concluded that these substances might have high affinity to the lipid membrane and be absorbed at the membrane surface. It implies that the increase in membrane resistance is not characteristic of bitterness always. This is supported by previous work, [9] where sucrose (sweet) increased the resistance of the lipid membranes made up of dioctyl phosphate (DOP), oleyl amine (OAm) and trioctyl methyl ammonium chloride (TOMA) while quinine (bitter) decreased the resistances of the above three lipid membranes. On the contrary, quinine increased the membrane resistance of the LB membrane. There is therefore room for further research to find out how exactly the membrane resistance from different lipid membranes change due to sweet and bitter substances.

The results also show that using this technique for distinguishing the two amino acids, the resistance changes with increasing concentration of the two amino acids are almost the same. This implies that there is need for precision right through the whole process (i.e., fabricating the membrane and taking measurements).

Results using different chiral materials for the lipid membranes (e.g., Span 80, Span 40) are going to be analysed and then the difference in the resistance change of the lipid membrane for the two amino acids will be more defined.

Of course, more work should be done on Span 20 itself which was used in this work. The work will confirm whether the reagent of Span 20 is made of L-Span only, D-Span only or LD-mixture, because no explicit description is made on this reagent. This will also help to clarify further the importance of chirality in the technique of impedance change of the lipid membrane.

6. Conclusion

Amino acid D-tryptophan was distinguished from L-tryptophan using the technique to measure the change of the lipid membrane impedance. The ability to separate the two amino acids may depend on the chirality of the membrane.

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