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Discrimination of D-Amino Acids from L-Amino Acids Using Electric Potential Changes of a Membrane

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Electric potential changes of optically active membranes due to different amino acid isomers were measured using a multichannel lipid membrane sensor. Because of the diastereomeric interaction between amino acids and the membrane, the membrane potential was determined by the optical activity of both the amino acid and the membrane. Diastereomeric interactions occur when an enantiomeric membrane resides in a chiral environment. The homochiral and heterochiral diastereomeric interactions differ sufficiently in the resultant arrangement of molecules at the surface of the membrane and permit the discrimination of D-amino acids from L-amino acids due to changes in the membrane characteristics.

1. Introduction

D-amino acids are optical isomers of L-amino acids. These isomers are mirror images of each other and are known as enantiomers. They have identical physical properties, except for the direction of rotation of the plane of polarized light. Although they have very close physicochemical similarities, enantiomers behave differently to biological bodies, *e.g.,* only D-tryptophan and D-leucine are sweet and only L-glutamic acid tastes umami and enhances the flavor of food. Amino acids are very important, mainly because protein molecules are made up of L-amino acid units. Proteins are found in every living cell and are the principal material of skin, muscle, tendons, nerves and blood.[1,2]

As mentioned above, amino acids possess different tastes and, from this point of view, amino acids with the same optical activity were discriminated using a multichannel taste sensor.[3] A multichannel potentiometric 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.[4–7] Although the taste sensor can discriminate between different amino acids according to their taste, it cannot discriminate two optical isomers even if they have different tastes.[8] Developing a sensor which can discriminate D-amino acids from L-amino acids will not only broaden their application, but the development of such a technique, to discriminate optical isomers using a simple sensing method, will be a breakthrough in the fields of chemical industry, medical science and pharmaceutics.

The membrane impedance change[8–13] of optically active membranes due to interactions between amino acids and the membrane itself was employed to discriminate D-amino acids from L-amino acids. Although the change in the membrane impedance corresponded to the optical activity of each respective amino acid, reproducibility was not very good because membrane resistance is difficult to measure with high precision and stability.

On the other hand, the membrane potential is easy to measure with high reproducibility using a multichannel taste sensor.[4–7] From the results of the membrane resistance changes for D- and L-amino acids,[10] it is supposed that a sufficient effect occurs in the membrane characteristics such that the occurrence of an appropriate reaction such as diastereomer formation results in a membrane potential change depending on the chirality of the amino acid. This work has been performed to investigate if the electric potential of an optically active membrane depends on diastereomeric interactions.

2. Detecting Principle, Materials and Methods

2.1 *Diastereomeric mechanism*

Diastereomers are also stereoisomers but are different from enantiomers because they have two or more chiral centers, they are not mirror images of each other, and two diastereomeric isomers have different physical properties. A bimolecular combination of two chiral substances might lead to the formation of four diastereomers only if the interaction does not destroy the chiral centers. Such interactions which promote the formation of diastereomers are known as diastereomeric interactions and are most common when the interaction involves optically active organic bases and organic acids to yield diastereomeric salts.

Figure 1 shows the application of a diastereomer formation interaction known as the resolution of racemic modifications, i.e., the separation of racemic modifications into enantiomers. A racemic modification is a mixture of equal parts of enantiomers and is optically inactive. Enantiomers making up the racemic modification have identical physical properties (*e.g.*, boiling points, relative densities, refractive indexes etc.) and hence cannot be separated by usual methods of fractional distillation or fractional crystallization.

As illustrated in Fig. 1, addition of an optically active base, L-Base, to a racemic acid (mixture of D- and L-Acid) will result in a mixture of two salts, [Diastereomer salt D-L] and [Diastereomer salt L-L] which are diastereomeric isomers of each other and can be

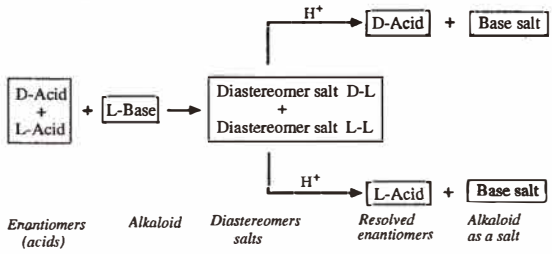

Fig. 1. Resolution of racemic modifications is a process by which enantiomers are separated by forming diastereomers which are subsequently separated using fractional crystallization.

separated by usual resolution procedures, *e.g.*, fractional crystallization and fractional distillation, since diastereomers have different physical properties. The optically active acids can then be recovered from each salt by addition of a strong mineral acid, which replaces the weaker organic acid.

The scheme in Fig. 2 shows that the membrane is prepared in such a way that its interaction with both D- and L-amino acids resembles the diasteremer formation reactions. When enantiomeric membranes reside in an optically active environment, homochiral and heterochiral diastereomeric interactions are created depending on the optical activity of the membrane and the environment.[2,14–16] These interactions might differ sufficiently in the arrangement of the molecules of the membrane surface to permit the discrimination of optical substances due to the changes in the membrane characteristics such as membrane potential and membrane impedance.[10]

2.2 *Making the membrane*

Because D- and L-amino acids are optical isomers, we used chiral substances to make the membrane. This allows both amino acids to alter the orientation of molecules at the surface of the membrane, consequently resulting in the alteration of the membrane's electric potential. Two different types of optically active membranes were prepared, and each membrane consisted of an optically active reagent. Polyvinyl chloride (PVC), the membrane forming matrix, was dissolved in tetrahydrofuran (THF); a chiral alkaloid, quinine (L), was used as the chiral reagent and was also dissolved in THF. Quinidine (D), which is an optical isomer of quinine, was used for comparison. Finally, the plasticizer was mixed with the PVC-optically active reagent-THF mixture resulting in the membrane solution. The two plasticizers used were trioctylmethylammonium chloride (TOMA), which gives rise to a positively charged membrane, and di-octyl phenyl phosphonate (DOPP), which gives a neutral membrane. The membrane solution was poured into a glass plate which was then placed on a hot plate with temperature controlled at 30°C to allow all the THF to evaporate, leaving behind the membrane as a colorless film of about 200 μm thickness. The membrane was then pasted on electrodes as shown in Fig. 3. The electrodes were made of Ag wire whose surface was plated with AgCl. The internal cavity was filled with 3 M KCl. The electrodes were dipped in the standard solution (1 mM KCl) for at least two days before measurements were taken to improve the stability of the membranes.

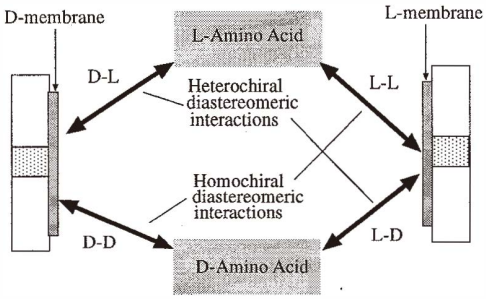

Fig. 2. Homochiral and heterochiral diastereomeric interactions depend on the optical relationship between the amino acid and the membrane.

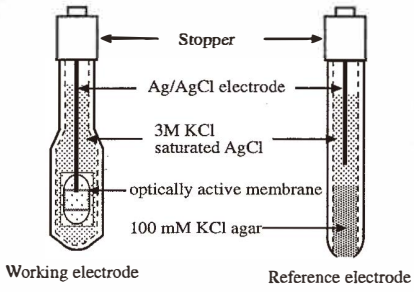

Fig. 3. The electrode, where the membrane is pasted, and the reference electrode.

2.3 *Multichannel sensor system*

Measurement of the membrane electric potential was done at room temperature using the recently developed multichannel taste sensor (S402 Anritsu Corp.) shown in Fig. 4. The working electrode where the membranes are pasted and the reference electrode are connected to the taste sensor. The membrane potential is the electric potential difference between the working electrode and the reference electrode.

The measurement cycle started with washing the electrodes by dipping them in and removing them from the standard solution for about 30 s. The electric potential of the membrane while in the standard solution was measured repeatedly until the change in the electric potential with respect to the preceding measurement was within ± 0.5 mV. The last value obtained was considered the electric potential of the standard solution. Once the standard solution values were determined, electrodes were immersed into sample solutions containing D- and L-amino acids at different concentrations. The difference between the sample electric potential and the standard solution represented the change in membrane electric potential due to each respective amino acid. The electrodes were washed again for 30 s before starting the same process for the next sample. One cycle was completed after

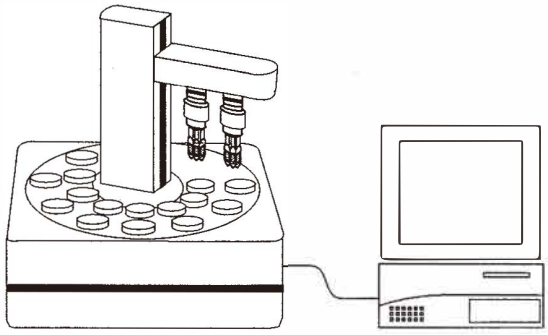

Fig. 4. Multichannel taste sensor.

measuring all the samples. The cycle was repeated four times and the obtained results, showing the averages and standard deviations, are summarized in the results section.

3. Results

The electric potential values, V_{ds}, for each sample were measured. Equation 1 shows an expression for V_{ds} where V_s and V_0 are the membrane electric potential when the membrane is immersed in the sample solution and when it is immersed in the standard solution, respectively.

$$V_{ds} = V_s - V_0 \tag{1}$$

The change in electric potential depending on the concentration of the sample, V_{dc}, was also determined. It represents the difference between the electric potential of the sample at a certain concentration, V_{ds}, and V_{d0}, which is the electric potential of the minimum concentration of the sample, in this case, 0 mM amino acid. Therefore V_{dc} can be expressed as follows:

$$V_{dc} = V_{ds} - V_{d0} \tag{2}$$

Figure 5 shows the membrane potential change due to tryptophan dissolved in 1 mM KCl solution. KCl solutions at very low concentration are usually used as standard solutions. Tryptophan samples prepared using 1 mM KCl showed potential change curves similar to those of samples prepared with pure water.

Unlike the quinine-DOPP membrane shown in Fig. 5(a), the quinine-TOMA membrane potential change decreases at a slightly higher rate for the case of L-tryptophan than that of D-tryptophan as shown in Fig. 5(b). Although the difference in responses between D- and L-tryptophan is statistically significant in the high concentration region, the difference is very small because of the weak diastereomeric interactions.

Figure 6 shows responses to glutamic acid dissolved in 1 mM KCl solution. D-glutamic acid increases the DOPP membrane potential at a higher rate than L-glutamic acid as shown in Fig. 6(a). Both D-glutamic acid and L-glutamic acid decreased the quinine-TOMA membrane potential, but the rate at which the potential decreases due to L-glutamic

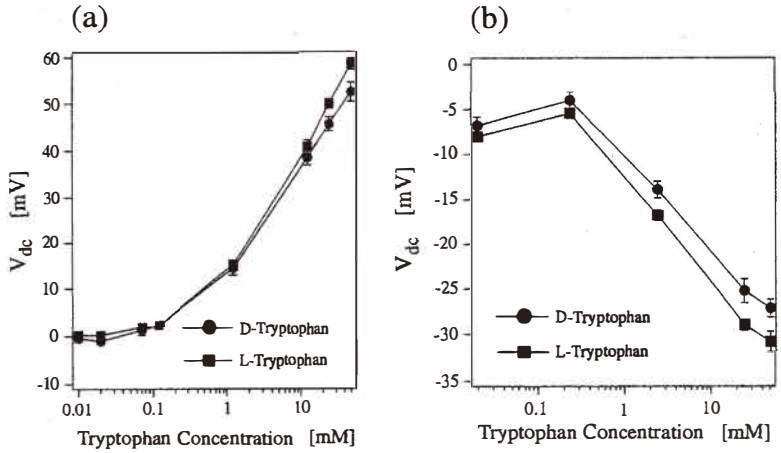

Fig. 5. The electric potential change due to tryptophan. (a) quinine-DOPP membrane and (b) quinine-TOMA membrane.

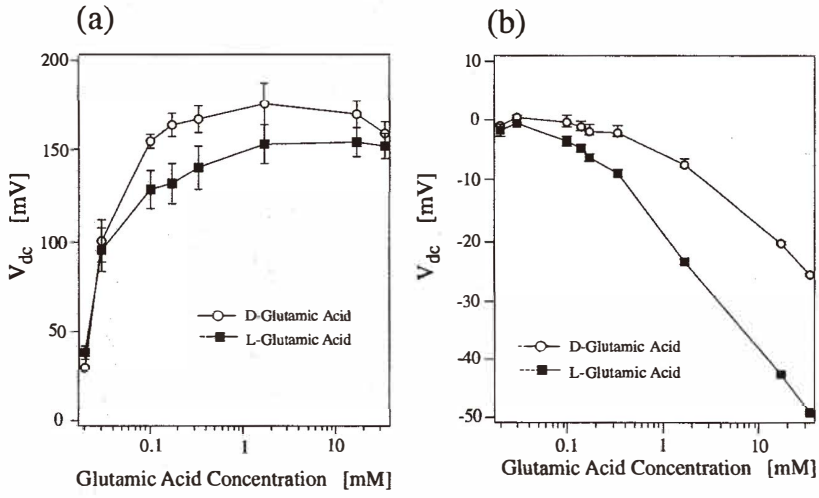

Fig. 6. The electric potential change due to glutamic acid. (a) quinine-DOPP membrane and (b) quinine-TOMA membrane.

acid is faster as shown in Fig. 6(b). This therefore implies that homochiral interactions (L-L) decreased the membrane potential more than heterochiral interactions (L-D). Although glutamic acid samples prepared using pure water and those prepared using 1 mM KCl solution had similar membrane potential change curves, the decrease in the membrane potential was significantly reduced for the case of glutamic acid samples prepared using 1 mM KCl. This is probably due to the fact that the interaction between quinine molecules

and amino acid molecules was weakened by the possible interaction of Cl^- ions with TOMA. The other reason might be that the dissociation of glutamic acid from quinine is likely since the interactions between the two chemicals were decreased by K^+ and Cl^-.

The change in the potential of the quinine-DOPP membrane for aspartic acid, just as for the case of glutamic acid, showed sharp increases at low concentrations as shown in Fig. 7(a). Figure 7(b) shows that L-aspartic acid dissolved in 1 mM KCl solution decreased the electric potential of the quinine-TOMA membrane at a rate higher than that of D-aspartic acid. Aspartic acid samples prepared using 1 mM KCl also showed a significant decrease in the change in the membrane potential, although the two optical isomers can still be discriminated as before.

Although the quinine membrane showed that it is possible to discriminate D-amino acids from L-amino acids, the quinidine membrane failed to distinguish the amino acids. This confirms the fact that the potential change was attributed to the diastereomeric interactions, because quinine is used in many resolutions of racemic modifications while quinidine is rarely used.

4. Discussion

Aspartic acid and glutamic acid contain a second carboxyl group and are known as acidic amino acids with pH values of 2.77 and 3.22, respectively. Since diastereomeric interactions are favorable between optically active acids and bases, there is a sharp increase in the membrane potential change at low concentrations of both glutamic acid and aspartic acid, as shown in Fig. 6 and Fig. 7, respectively. In both cases, D-amino acids were clearly discriminated from L-amino acids when V_{dc} reached the saturation level. This implies that the threshold concentration for V_{dc} increment for both glutamic acid and aspartic acid

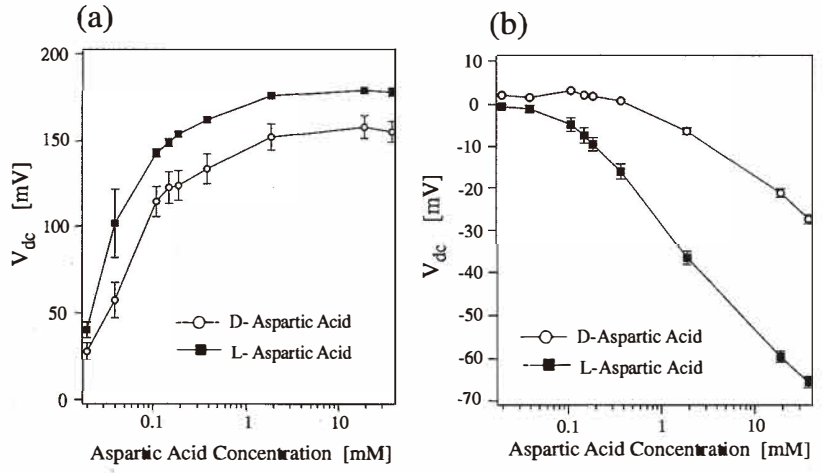

Fig. 7. (a) The electric potential change due to aspartic acid: (a) quinine-DOPP membrane and (b) quinine-TOMA membrane.

mainly depends on the pH values of the amino acids. This was not evident in the case of an amino acid with weaker acidity, *e.g.*, tryptophan (Fig. 5), which has a pH value of 5.89. This confirms that diastereomeric interactions occurred between the acidic amino acids and the membrane and that D- and L-tryptophan could not be distinguished by this membrane with high sensitivity.

Diastereomeric interactions between amino acids and the alkaloid (quinine) might not have been as strong as those between alkaloids and other carboxylic acids. Although amino acids do have the carboxyl group, certain properties are not consistent with this structure, *e.g.*, glycine has an acidity constant of $K_a = 1.6 \times 10^{-10}$ whereas most carboxylic acids have K_a values of about 10^{-5}.

5. Conclusions

Diastereomeric interactions between the optically active membrane and amino acids caused different membrane electric potentials allowing D-amino acids to be discriminated from L-amino acids. The results obtained pave the way for further research on the development of a chemical sensor for optically active substances. Among other applications, a chemical sensor may use the membrane potential change effect to distinguish between optical isomers and to measure the optical purity of optically active substances.

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