대한임상검사학회지 : 36권 제2호, 115-120, 2004

p-Dimethylaminobenzaldehyde, 1-Naphtol, Sulfosalicylic acid 등의 Carrier를 함유하는 H_2O-CH_2Cl_2-H_2O Liquid Membrane을 이용한 아미노산의 선택적 분리(II)

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Selective Separation of Amino Acid Mixture Using H_2O-CH_2Cl_2-H_2O Liquid Membrane containing p-Dimethylaminobenzaldehyde, 1-Napthol and Sulfosalicylic acid as a Carrier (II)

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A bulk liquid membrane system was introduced for selective separation of an amino acid mixture. We confirmed p-diamethylaminobenzaldehyde (DAB), sulfosalicylic acid (SSA) and 1-naphtol were very useful carriers for selective separation of an amino acid mixture. As a result, Ala, Leu, Val, Phe and Ile were successfully separated by SSA, 1-naphtol in basic condition, 1-naphtol in weak acidic condition, DAB in strong acidic condition and DAB in strong basic condition. The separation mechanism was proposed by ion pair mechanism in the case of SSA and 1-naphtol and Imine bond formation mechanism was also introduced for DAB.

Key Words : Liquid membrane, p-Diamethylaminobenzaldehyde, Sulfosalicylic acid, 1-Naphtol, Selective separation, Amino acid

I. INTRODUCTION

An amino acid is an essential constituent of protein polymer back bone and has many biological functions. For instance, the α-amino acid and its derivatives, amino butyric acid, serotonin and melatonin act as a stimulant in the nervous system. Thyroxine and indoleacetic acid have similar functions as a hormone. Amino acids containing the nitrogen atom are also basic compounds constructing other important biological compounds like nucleotides, hem and chlorophyll. Arginine, citrulline and ornithine are metabolic intermediates. As shown above, amino acids have many important roles in clinical and pathological fields. In this study, we introduced the liquid membrane system for separating an amino acid mixture which has many important roles in biological chemistry.

Liquid membranes are usually classified in three cases

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abbreviated "SLM" (supported liquid membrane), "ELM" (emulsion liquid membrane) and "BLM"(bulk liquid membrane), Fig. 1. To provide thin layer liquid membranes, SLM consists of a polymeric filter, known as immobilized liquid membrane (supported liquid membrane) (Kesting, 1985). In 1984, the standard of selectivity for SLM was studied (Noble, 1984). In 1987, Danesi, Reichley-yinger and Rickert co-related the interactions of surface tension, water and carrier (Danesi et.al., 1987).

"ELM" is an abbreviation for Emulsion Liquid Membrane. The source and receiving phase are emulsified to transfer the water soluble sample into the hydrophobic membrane phase. In 1982, Marr and Kopp built a guideline for water-in-oil ELM system(Marr and Kopp, 1982). The most important problem for ELM is the destruction of this system by large osmotic pressure differences derived by internal metal ion concentration (Draxler and Marr, 1986).

BLM (bulk liquid membrane) has relatively thick layer so the permeability is not good. To prevent this phenomenon U-tube cell is devised as shown in Fig. 2. The stirring module is equipped to improve the permeability.

Christensens used the H_2O-CH_2Cl_2-H_2O liquid membrane system to separate various cations with macrocyclic polyethers as a carrier (Christensen, 1987). McBride studied alkali earth metasl and Pb^{2+} ion separation with the similar system described above--especially the H_2O-$CHCl_3$-H_2O liquid membrane system with macrocyclic polyethers as a carrier (McBride jr and Christensen, 1984). In 1985, Wiltold used Poly Sodium Sulphonate Membrane for metal cation separation. (Wislawa and Wiltold, 1985). In 1986, Izatt and Jones separate Hg^{2+}-$M^{N+}$by using a 1M HNO_3-$CHCl_3$-HNO_3 liquid membrane system (Izatt and Jones, 1986).

In this study, BLM system based on the H_2O-CH_2Cl_2-H_2O liquid membrane is used to separate amino acid mixture using p-diamethylaminobenzaldehyde(DAB), sulfosalicylic acid(SSA) and 1-naphtol as a carrier.

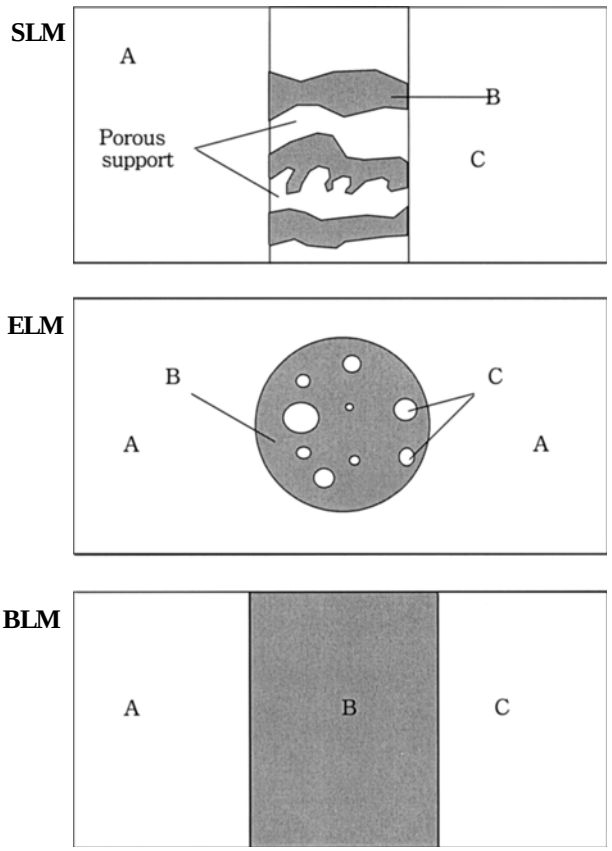


Fig. 1. Comparison of Supported Liquid Membrane (SLM), Emulsion Liquid Membrane(ELM) and Bulk Liquid Membrane (BLM) Systems. (A is the source (feed) phase, B is the liquid membrane and C is the receiving phase.)

II. MATERIALS AND METHOD

Membrane transport experiments were carried out using bulk liquid membranes. Each cell consisted of 3.0mL membrane phase (CH_2Cl_2 1.0mM in carrier, stirred at 120 rpm by a magnetic stirrer) interfaced to both a 0.8 mL source phase and 5.0 mL receiving phase. 1.0 M amino acid aqueous solutions prepared for each Ala, Gly, Gln, Val, Leu, Ile, Cys, Phe and His. This was mixed together and made it up to 1.0 mL with equal amount. The mixed amino acid solution was injected into source phase with a syringe. After 12 hrs at room temperature, the sampling was carried out by a capillary from the receiving phase and TLC is used for the identification of the separated amino acid. The Rf value was examined for n-BuOH, AcOH, H_2O(4:1:1) and the coloring agent was $KMnO_4/K_2CO_3$ aqueous solution.

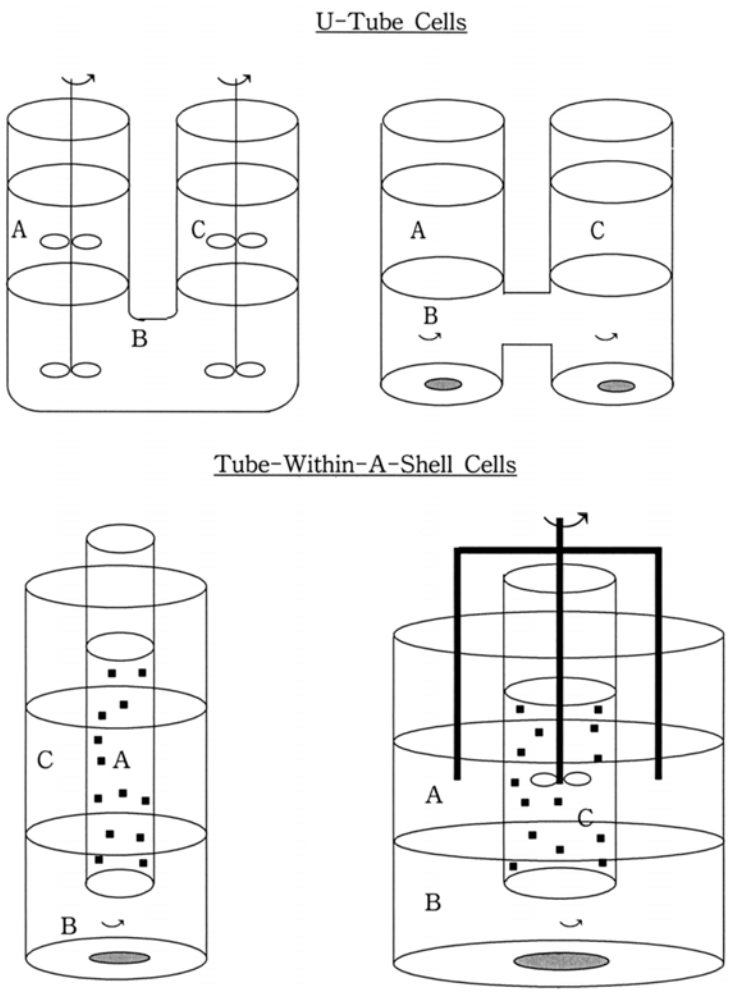


Fig. 2. Four types of cells used to study transport across bulk liquid membranes.
(A is the source (feed) phase, B is the liquid membrane and C is the receiving phase.)

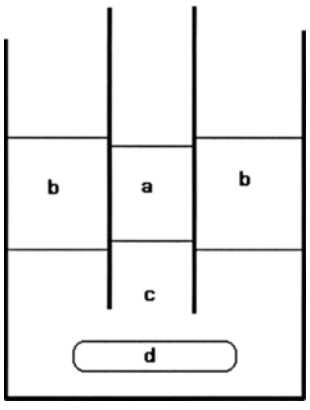


Fig. 3. Separation apparatus. (b is the source (feed) phase, c is the liquid membrane, a is the receiving phase and d is the magnetic bar.)

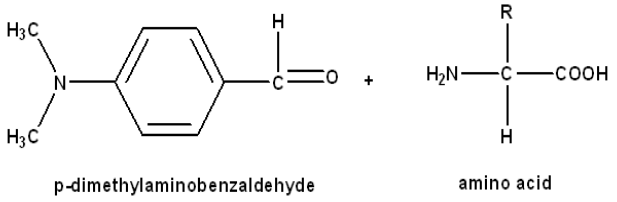
III. RESULT AND DISCUSSION

As shown above, Ala, Leu, Val, Phe and Ile were successfully separated by SSA, 1-naphtol in basic condition, 1-naphtol in weak acidic condition, DAB in strong acidic condition and DAB in strong basic condition(Table 1.).

Table 1. Overall separation data

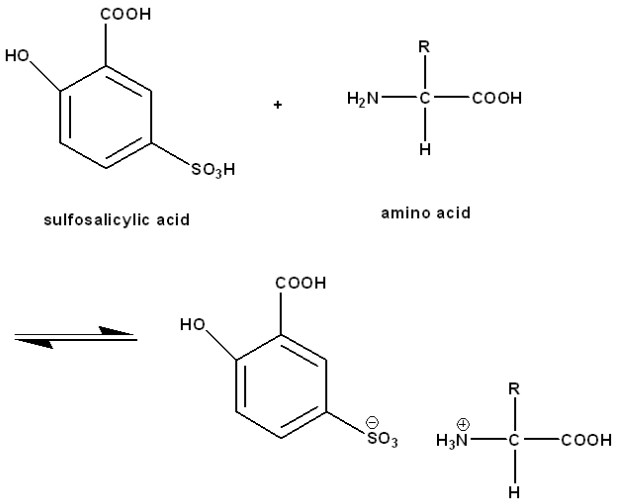
run#	a	b	c	spot	Rf	acid and base
1	Val	mixture(9), PVA/1-naphtol	CH_2Cl_2	1	0.36	0
2	Val	mixture(9), PVA/1-naphtol	CH_2Cl_2	1	0.31	AcOH
3	Leu	mixture(9), PVA/1-naphtol	CH_2Cl_2	1	0.51	NH_4OH
4	Leu	mixture(9), PVA/1-naphtol	CH_2Cl_2	1	0.5	NaOH
5	Ile	mixture(9), PVA/1-naphtol	CH_2Cl_2/BuOH	1	0.42	NH_4
6	Ala	mixture(9), PVA/SSA	CH_2Cl_2/BuOH	1	0.2	NaOH
7	Ala	mixture(9), PVA/SSA	CH_2Cl_2/toluene	1	0.24	NH_4OH
8	Ala	mixture(9), PVA/SSA	CH_2Cl_2/toluene	1	0.28	NaOH
9	Ala	mixture(9), PVA/SSA	CH_2Cl_2/BuOH	1	0.23	NH_4OH/NaOH
10	Ile	mixture(9), DAB	CH_2Cl_2	1	0.4	0
11	0	mixture(9), DAB	CH_2Cl_2	0	0	AcOH
12	Phe	mixture(9), DAB	CH_2Cl_2	1	0.46	HCl
13	Ala, Val Ile	mixture(9), DAB	CH_2Cl_2	3	0.24 , 0.37, 0.43	NH_4OH
14	Ile	mixture(9), DAB	CH_2Cl_2	1	0.4	NaOH

As shown in the Scheme 1. p-dimethylaminobenzaldehyde(DAB) can make imine bond with amino acid.

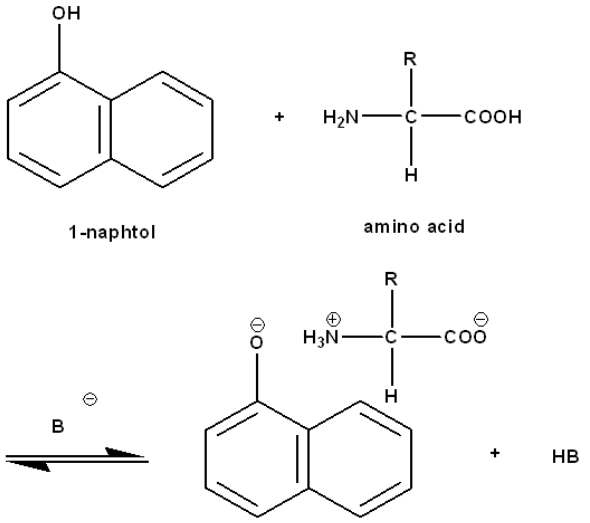


Scheme 1. Reaction scheme of forming imine bond.

In the case of Sulfosalicylic acid (SSA), ion-pair can be confirmed as shown in the Scheme 2. And 1-naphtol also shows ion-pair formation mechanism in the Scheme 3.

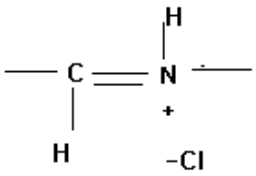


Scheme 2. Ion pair formation reaction for SSA with amino acid.



Scheme 3. Ion pair formation for 1-naphtol in the basic condition.

The interpretation of data, run# 10~14 of DAB is explained by the mechanism, Scheme 1. DAB is acting as a carrier in acidic, basic and neutral condition as a promoter leading the fore-going reaction. In the weak acid condition, the imine is stabilized by acetic acid. The strong intensity on the TLC plate in the strong acidic condition is explained by the consideration of the stabilization mechanism, Scheme 4. Three spots in weak basic condition means low selectivity correspond to low stability of imine bond.



Scheme 4. Ammonium salt formation.

Run# 6~9, data of SSA shows very dramatic selectivity for Ala. This phenomena is considered as an effect of ion-pair mechanism. The stability of ion pair is strongly dependent on the size of amino acid substrate. The smaller size of amino acid gives the ion-pair mechanism greater stability, Scheme 2.

Run# 1~5, data of 1-naphtol shows more selectivity than SSA and still more study is needed to explain this.

The identification for separated amino acid is carried out on the basis of reference data.(Table 2.)

Table 2. Rf-value for various amino acid in IPA, H2O (9:1)

amino acid	Rf-value
Leu	0.48
Phe	0.45
Ile	0.42
Val	0.33
Ala	0.21
Gln	0.15
Gly	0.12
His	0.09
Cys	–

IV. CONCLUSION

1. DAB(p-diamethylaminobenzaldehyde) acts as a carrier in acidic, basic and neutral conditions.
2. DAB(p-diamethylaminobenzaldehyde) shows very good permeability in strong acidic conditions rather than basic conditions.
3. DAB(p-diamethylaminobenzaldehyde) can't act as a carrier in a weak acidic condition.
4. DAB(p-diamethylaminobenzaldehyde) shows low selectivity in weak basic conditions showing three spots.
5. SSA(sulfosalicylic acid) has special selectivity for Ala.
6. 1-naphtol separates Val in neutral and weak acidic conditions.
7. 1-naphtol separates Leu selectively in basic conditions.

Thus, We have referred to the benefit from the selective separation of amino acid mixtures in human samples using the liquid membrane.

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혼합 amino acid의 선택적 분리를 위해 bulk liquid membrane system을 이용하였으며, p-diamethylamino-benzaldehyde(DAB), sulfosalicylic acid (SSA) , 1-naphtol이 amino acid의 선택적 분리를 위한 효과적인 carrier로 사용될 수 있음을 확인하였다. 그 결과 sulfosalicylicacid에 대해서 Ala에 대한 선택성을 관찰할 수 있었으며, 1-naphtol에 대해서는 염기성 상태에서 Leu을 약산성 및 중성상태에서는 Val을 선택적으로 분리할 수 있었다. DAB에 대해서는 강산성에서 Phe을, 강알카리 조건에서 Ile를 선택적으로 분리할 수 있었다.

Separation mechanism은 SSA와 1-naphtol의 경우에는 ion pair mechanism으로, DAB의 경우에는 imine 결합의 생성반응으로 설명할 수 있었다. 따라서, Liquid membrane을 이용한 생체시료 내의 아미노산을 선택적으로 분리함에 유용성이 클 것으로 사료된다.