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Extraction and carrier-facilitated transport of amino acids using synthetic non-cyclic receptors through bulk liquid membrane

Pratibha Joshi, Nidhi Joshi and Uma Sharma*

School of Studies in Chemistry, Vikram University, Ujjain (MP) 456 010, India

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The extraction and carrier-facilitated transport of amino acids (leucine, valine and glycine) was studied through chloroform bulk liquid membrane system using a series of non-cyclic receptors such as diethylene glycol (1), diethylene glycol dimethyl ether (2), diethylene glycol dibutyl ether (3), diethylene glycol dibenzoate (4), triethylene glycol (5) and tetraethylene glycol (6). The amount of amino acid extracted and transported depends mainly upon the structure and the concentration of the receptors and also on the concentration of amino acid. The receptors 1 to 4, having small chain length and flexible end groups, formed stable complexes with amino acids, and the flexibility of receptors in different conformational forms was responsible for their carrier ability, while the receptors 5 and 6, having larger chain length showed poor carrier ability. Hydrophobicity of amino acids also play an important role in the extraction as well as transport process.

Keywords: Extraction, Liquid membrane, Carrier-facilitated transport, Receptors.

Interaction and transport of amino acids by synthetic receptors is a topic of current interest in biochemical processes[1]. The amino acid dehydrogenases can be used in biosensor diagnostic kits to screen blood serum for elevated level of free amino acids associated with certain typical types of diseases[2,3]. Macrocyclic receptor molecules like crown ether, cryptands and podands have been utilized for the selective transport of amino acids through bulk and supported liquid membrane[4,5]. The liquid membrane systems have much importance as models for transport of neutral species across biomembranes. They are helpful in understanding complex behaviour of biochemical transport processes and provide us the useful information about structural unit of receptors that affects transport.

*Author for correspondence
E.mail: umasharma10@rediffmail.com
Fax: 91-734-2514276

In the present study, we have investigated the extraction and carrier-facilitated transport of amino acids (leucine, valine and glycine) through chloroform bulk liquid membrane system using a series of non-cyclic synthetic receptors (Fig. 1).

Materials and Methods

Experimental

The non-cyclic receptors namely diethylene glycol dimethyl ether, diethylene glycol dibutyl ether, diethylene glycol dibenzoate, diethylene glycol, tri-ethylene glycol and tetraethylene glycol were obtained from Fluka & Co., Switzerland. Amino acids (glycine, leucine and valine) were obtained from Lanchastar, Chennai, India. The solvents were used after distillation. Perkin-Elmer Lambda EZ 201 UV/vis spectrophotometer was employed for the estimation of amino acids.

Extraction studies

For extraction studies[6], the equal volume (10 mL) of aqueous solution of amino acid concentration (0.1 *M* to 3.0 *M*) and the receptor solution concentration (0.1 *M*) in chloroform were vigorously stirred in beaker for 24 h on a magnetic stirrer. The beaker was covered and kept in a thermostatic water bath (25° ± 1°C). After stirring, the mixture was allowed to stand for 5 min for the separation of two phases. The depleted aqueous phase was removed and analyzed for amino acids using Perkin-Elmer UV/vis spectrophotometer. The amount of amino acid extracted by receptors was determined by its difference in aqueous

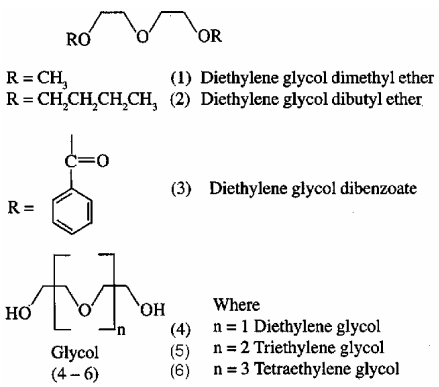

Fig. 1—Receptors used in study

phases, before and after extraction. The distribution ratios were calculated as follows[7]:

$$D_M = \frac{\text{Total conc. of amino acid in organic phase}}{\text{Total conc. of amino acid in aqueous phase}} \quad \ldots (1)$$

Transport studies

Transport experiments[8] were performed in a U-tube glass cell, placed in a thermostatic water bath (25° ± 1°C). The 0.1 *M* receptor in 25 mL of chloroform was placed in the bottom of the U-tube served as the membrane. The 10 mL of aqueous amino acids solution of concentration 0.1 *M* to 3.0 *M* was placed in one limb of the U-tube to serve as source phase and 10 mL of double-distilled water was placed in another limb, which served as the receiving phase. The membrane phase was constantly stirred using magnetic stirrer. The samples were withdrawn from source and receiving phases, after 24 h and analyzed for amino acids transported using Perkin-Elmer UV/vis spectrophotometer. Amino acid fluxes were calculated by using the relation[9]:

$$J_M = \frac{C_{(\text{receiving})}\, V}{A(t)} \quad \ldots (2)$$

where C was the concentration of amino acids in receiving phase (mol/dm^3), V, the volume of receiving phase (dm^3), A, the effective area of membrane (m^2) and t, the time in s.

Estimation of amino acids

The 0.5 mL of aqueous amino acid solution (0.1 *M*) was taken in 10 mL standard volumetric flask and the 0.2 mL ninhydrin solution (0.5%) was added and kept the flask in the boiling water for 10 min[10]. Thereafter, the 6 mL of 60% ethanol was added with double distilled water until the total volume was 10 mL. The λ_{max} of this solution was found at 575 nm by Perkin Elmer spectrophotometer. Calibration curve was obtained for different concentrations of amino acids, which was used for the estimation of amino acids in the source and receiving phases.

Results and Discussion

The results of extraction and transport studies of amino acids with receptors 1 to 6 through $CHCl_3$ bulk liquid membrane after 24 h are reported in Tables 1 and 2. The blank experiments were performed with different concentrations of amino acid in which the membrane contained no carrier. No detectable amount of amino acid across $CHCl_3$ membrane could be observed in receiving phase in the blank experiments, which proved that there was no leakage. All measurements were performed in duplicate.

In order to find out optimum concentration for extraction and transport study, we varied the concentration of amino acids from 0.1 *M* to 3.0 *M*. As higher concentration solutions of amino acids were difficult to prepare and below 0.1 *M* concentration, no extraction and transport was observed, hence concen-

Table 1—Amount of amino acid extracted into an organic phase after 24 h with receptors 1 to 6 in chloroform

[Source phase: Amino acid solution (10 mL); Organic phase: 0.1 M solution of receptor in chloroform (10 mL)]

Conc. of amino acid	Receptors											
	1		2		3		4		5		6	
	Amount of amino acid extracted ($\times10^{-3}M$)	D_u	Amount of amino acid extracted ($\times10^{-3}M$)	D_u	Amount of amino acid extracted ($\times10^{-3}M$)	D_u	Amount of amino acid extracted ($\times10^{-3}M$)	D_u	Amount of amino acid extracted ($\times10^{-3}M$)	D_u	Amount of amino acid extracted ($\times10^{-3}M$)	D_u
Glycine												
0.5 M	46.50	0.2490	100.50	0.6870	98.75	0.6519	74.50	0.5963	45.00	0.1946	48.50	0.2139
1.0 M	77.00	0.6948	117.00	1.8825	102.50	1.1694	79.75	0.7442	59.25	0.5648	66.75	0.5679
2.0 M	64.00	0.4806	85.75	0.7351	82.75	0.7345	71.00	0.6928	58.00	0.3948	43.50	0.4514
Leucine												
1.0 M	297.50	2.8659	355.00	3.4738	337.50	2.9896	310.00	2.9006	237.50	2.0883	245.00	2.1510
2.0 M	562.50	1.9903	630.00	2.8054	610.00	2.4913	590.00	2.2235	517.50	1.4209	502.50	1.5314
3.0 M	742.50	1.5704	857.50	2.4133	812.50	2.0254	782.50	1.839	732.50	1.1486	647.50	1.5125
Valine												
1.0 M	387.50	2.8906	475.00	3.5126	455.00	2.9959	417.50	2.9573	377.50	2.3589	365.50	2.2826
2.0 M	627.00	2.0043	705.00	2.9315	690.00	2.5344	660.00	2.3598	615.00	1.9083	617.50	1.6806
3.0 M	795.00	2.0003	935.00	2.5863	912.50	2.4122	867.00	2.3003	757.50	1.8642	742.50	1.6529

Table 2 —Amount of amino acid transported into an organic phase after 24 h with receptors 1 to 6 in chloroform

[Source phase: Amino acid solution (10 mL); Membrane: Receptor solution in chloroform (25 mL); Receiving phase: Distilled water (10 mL)]

Conc. of amino acid	Receptors 1		2		3		4		5		6	
	Amount of amino acid trans-ported ($\times10^{-3}$M)	J_M ($\times10^{-6}$) (mol/m^2/s)	Amount of amino acid trans-ported ($\times10^{-3}$M)	J_M ($\times10^{-6}$) (mol/m^2/s)	Amount of amino acid trans-ported ($\times10^{-3}$M)	J_M ($\times10^{-6}$) (mol/m^2/s)	Amount of amino acid trans-ported ($\times10^{-3}$M)	J_M ($\times10^{-6}$) (mol/m^2/s)	Amount of amino acid trans-ported ($\times10^{-3}$M)	J_M ($\times10^{-6}$) (mol/m^2/s)	Amount of amino acid trans-ported ($\times10^{-3}$M)	J_M ($\times10^{-6}$) (mol/m^2/s)
Glycine												
0.5 M	44.25	22.65	99.50	50.94	94.50	48.38	65.50	33.53	42.00	2.50	43.25	22.14
1.0 M	97.25	49.79	112.25	57.47	86.75	44.41	67.25	34.43	69.50	35.58	50.24	25.72
2.0 M	56.25	28.80	72.75	37.34	76.75	39.29	72.50	37.12	42.50	21.76	41.50	21.24
Leucine												
1.0 M	262.50	134.40	337.50	172.80	330.00	168.96	300.00	153.60	230.00	117.76	235.00	120.32
2.0 M	532.50	272.64	622.50	318.72	602.50	308.48	562.50	288.00	497.50	254.72	487.50	249.60
3.0 M	699.35	358.06	852.50	436.48	796.85	407.98	764.35	391.34	641.85	328.62	612.50	313.60
Valine												
1.0 M	377.50	193.28	455.00	232.96	400.00	204.80	387.50	198.40	347.50	177.92	355.00	181.76
2.0 M	715.00	366.08	785.00	401.92	757.00	387.58	742.50	380.16	705.00	360.96	707.00	361.98
3.0 M	757.50	387.58	897.00	459.26	872.00	446.46	797.50	408.06	705.00	360.96	730.00	373.76

trations between 0.1 *M* to 3.0 *M* were used in the study. The receptor concentration was kept constant at 0.1 *M*. Optimum concentration of glycine was observed at 1.0 *M* and of leucine and valine at 3.0 *M*, for extraction and transport studies. In order to find out optimum concentration for receptors, the concentration of receptors was varied from 1.0×10^{-3} *M* to 0.3 *M*. The maximum amount of amino acids extracted as well as transported was at the 0.1 *M* concentration of receptor. The sequence of extraction abilities of the receptors for glycine and leucine was 2 > 3 > 4 > 1 > 6 > 5 and for valine the order was 2 > 3 > 4 > 1 > 5 > 6. The sequence of transport abilities of the receptors for glycine was 2 > 1 > 3 > 4 > 5 > 6 and for leucine and valine, the order was 2 > 3 > 4 > 1 > 6 > 5, respectively.

The structure of the receptor plays an important role in extraction and transport processes[11]. The receptors 1 to 4 possess the same dioxyethylene chain with different end groups and receptors 5 and 6 have the same end groups with tri- and tetraoxyethylene chain length. These receptors interact with amino acids by the hydrogen bonding between the NH_2 function of amino acids with the oxygen atom of the receptors[12] and this interaction is responsible for the extraction and transport of amino acids. Receptors (1-4) having small chain lengths with flexible end groups formed stable complexes with amino acids. The flexibility of receptors in different conformational forms is responsible for their carrier ability, while the receptors having larger chain length with rigid end groups show poor carrier ability[13]. Among all the receptors, receptor 2 showed the maximum carrier ability for amino acids, due to the presence of additional donor sites of the end group with small chain length.

The hydrophobicity of the amino acids also play an important role in the extraction and transport processes[14]. The sequence of hydrophobicity of amino acid was valine = leucine > glycine. The maximum amount of glycine was extracted and transported at 1 *M* concentration and decreased with the increase in initial concentration. In the case of leucine and valine, the amount of extraction and transport increased with the increase in concentration of leucine and valine (Tables 1 and 2). As the hydrophobicity of the glycine is lower, its tendency to pass from aqueous to organic phase was slow. But, in the case of leucine and valine, the distribution from aqueous to organic phase was high, due to higher hydrophobic nature of the molecule, so the maximum extraction and transport occurred above 1 *M* concentration.

In conclusion, the results showed that the amount of amino acid extracted and transported depends mainly upon the structure of the receptors, their concentration and also on the concentration and hydrophobicity of the amino acids.

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