Stereoselective Transport of Amino Acids and Peptides through Liquid Membranes*

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Received 11 April 2001

A short review concerning the possibility of stereoselective transport of amino acids, peptides, and their derivatives through liquid membranes is presented. The main scope of this paper is to show the variance of such approach and some difficulties in designing of suitable liquid membranes. The detailed discussion of transport mechanisms and carrier molecule architecture necessary to realization of enantioselective differentiation of amino acids, their derivatives, and peptides is also provided. Two different approaches for this type of separation are described and evaluated. They are as follows: application of chiral organic phase and the use of stereoselective receptors for amino acids and other related compounds transportation.

It is widely known that almost every biochemical process occurring in the cells of all living organisms is based on some specific, stereoselective interactions between reacting molecules. Therefore, if it were our intention to influence the course events, it would be very important to be concerned in the stereochemistry of molecules involved in those processes. This idea caused the rapid growth of whole branches of scientific activity dealing with the stereochemistry. As a consequence, almost all newly designed and biologically active substances, such as drugs or pesticides, are compounds with strictly defined stereostructure. One of the most important groups of the substances in which stereochemistry plays very significant role in the nature are amino acids, their derivatives, and peptides. Therefore, there is a great interest in obtaining those compounds with the required enantiopurity. It can be achieved by different approaches, *e.g.* stereoselective synthesis, biotransformation or chiral separation. Secondly, the stereoselective interactions of amino acids with other molecules are not fully recognized yet and there is a great pressure to develop the methods that can help in understanding them.

Among various accessible techniques one can point out membrane separation as the most perspective method of separation. Different types of membranes and membrane processes were applied for amino acids enantiomers discrimination. One can point out the application of membranes obtained from molecularly imprinted polymers (MIP) [1], enhanced ultrafiltration with proteins [2] or chiral micelles [3] as discriminating factors, plasma-polymerized membranes [4, 5], and liquid membranes (LM). Here liquid membranes can be used as chiral separators or as a convenient tool to examine the nature of stereoselective interaction with chiral carriers. Moreover, liquid membranes can be used as a screening method for the preliminary active survey of amino acids receptors for other purposes.

In general, the liquid membrane can be defined as a selective liquid barrier between two semicontinuous phases. They are called donor (source or feed) and acceptor (receiving or stripping) phases [6]. Commonly, organic solvent forms liquid membrane and donor and acceptor are aqueous solutions, but the reverse arrangement was also reported [7]. Three main types of liquid membranes are known today. They are as follows: bulk liquid membranes (BLM), emulsion liquid membranes (ELM), and supported liquid membranes (SLM) [6]. They differ in the method of preparation, surface area, and thickness. BLM can be realized *e.g.* in U-tube where heavier organic liquid is placed on

*Presented at the 28th International Conference of the Slovak Society of Chemical Engineering, Tatranské Matliare, 21—25 May 2001.

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the bottom and two aqueous phases are in the arms of tube. ELM is made up of water/oil/water double emulsion system stabilized by addition of surfactant to the organic, liquid phase. Finally, SLM can be obtained by soaking of support – porous polymeric membrane – with organic, liquid phase. All types of liquid membranes were successfully applied for separation of such organic substances as: organic acids [8], amines [9], amino acids [10], and other biologically active compounds [11].

The main goal of this review is to present the state-of-art for amino acid and peptides stereoselective separation by means of liquid membranes. The subject is discussed in terms of possible separation of amino acids and their derivatives into enantiomers. Additionally, the transport of short peptide diastereoisomers is also presented. Two methods of separation are considered. They are application of chiral liquid phase as the membrane and the use of various stereoselective carriers dissolved in organic phase. Moreover, the mechanism for transport of amino acids enantiomers and the character of interactions responsible for molecular recognition are also discussed.

AMINO ACIDS AND PEPTIDES TRANSPORT THROUGH LIQUID MEMBRANES – TYPES AND MECHANISMS

Before further discussion about the potential of stereoselective separation of amino acids or peptides with liquid membranes, it is necessary to put forward some general comments on the nature and the properties of such process. The authors' intention is to show the diversity of optical isomers transport and some aspects of its realization. The authors also believe that this survey should give some guidelines for rational design of a particular liquid membrane system.

Amino acids and peptides are multicharged compounds that bear at least two oppositely charged functional groups: amine and carboxylic. As a consequence, those substances are positively or negatively charged in aqueous solutions at all pH with the exception of their isoelectric point. Simultaneously, the transfer of substance from aqueous phase to organic, liquid membrane can only occur if the molecule is uncharged. To meet this condition and enable amino acid or peptide to pass over organic liquid membrane several methods can be distinguished. The first approach appeals to change amino acid (or peptide) into derivative that has only one charged group and then, by proper pH adjustment, to convert amino acid into its uncharged form. The other possibility would be a selection of ion-pair reagent, dissolved in source, aqueous phase that interacts with amino or carboxylic groups. And finally, the third way is the introduction of the carrier into membrane phase able to complex and enhance the transport of the target molecule through organic phase. Of course, a combination of the above methods is also possible.

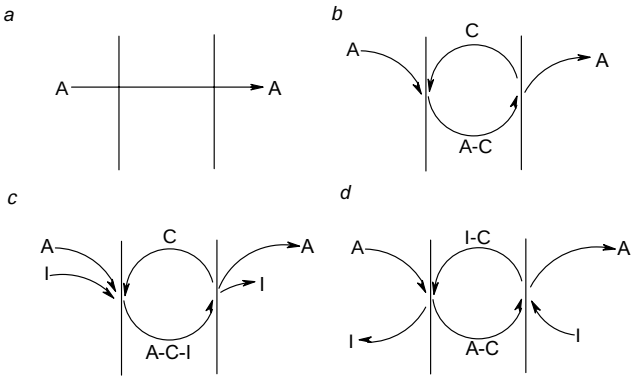


Fig. 1. Basic types of liquid membranes transport. *a*) Simple diffusion, *b*) facilitated diffusion, *c*) co-transport, *d*) counter-transport. A – amino acid, C – carrier, I – co- or counter-ion.

It is obvious that various kinds of transport modes in the liquid membranes follow different mechanisms. In two cases mentioned firstly, the transport mechanism is simply diffusion transport (passive) where a driving force is constituted by concentration gradient of the transported compound from donor to acceptor phase (Fig. 1). When the carrier is applied in the liquid membrane (active transport), there are two possible transport mechanisms depending on the nature of carrier regardless the ionic form of amino acid (or peptide) in donor phase. In case when neutral carrier complex transports guest (amino acid) molecule together with its co-ion across liquid membrane the co-transport mechanism takes place. The driving force of the process is the concentration gradient of co-ion between donor and acceptor phase. When carrier molecule bears ionizable groups the counter-current transport mechanism occurs. It means that the driving force of the process is counter-ion concentration gradient between acceptor and donor phase. Therefore, amino acid is transported against its concentration gradient.

STEREOSELECTIVE TRANSPORT OF AMINO ACIDS THROUGH LIQUID MEMBRANES – GENERAL CONSIDERATIONS

To develop transport stereoselectivity it is indispensable to create chiral environment in the system. It can be achieved in two manners, namely by an application of chiral organic liquid as a membrane phase or by introducing a chiral carrier into an achiral membrane.

Application of chiral liquid membrane for stereoselective separation was scarcely reported, so it will be discussed a little further in the text. Much more

frequently the use of chiral carrier was reported and thus it is reasonable to present some general remarks about the nature of such molecule.

Considering the carrier structure it is possible to apply several types of complexing molecules that can transport amino acid and peptide. The carrier molecule, independently of the guest ionic form, has to meet several requirements in order to stereoselectively recognize complexing molecule.

1. It should possess the structurally well-defined shape and be able to interact with guest molecule in such a manner that it ensures the stereospecific recognition of isomers. It is important to mention here that such molecular recognition can be gained by different type of weak, noncovalent molecular interactions. They can be rated as electrostatic (ionic, charge-dipole), hydrogen-bonding, electron donor-acceptor, van der Waals (repulsions, π—π electrons stacking), and hydrophobic interactions. The architecture of carrier molecule has to be, in the perfect case, complementary in geometry, charge distribution, size and nature of interacting points (or surfaces) to the guest molecule (in case of amino acids it should recognize all three parts – charged groups and side chain of compound) [12].

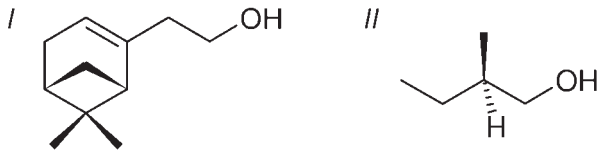
2. In case of facilitated transport it must be also pointed out that from one side the carrier molecule should obviously bind the amino acid strong enough to transfer it from aqueous phase to liquid membrane, by compensating the energetic desolvation costs of bringing hydrate polar groups out from aqueous environment. From the other side, however, those interactions have to be weak enough to allow releasing of transported substance from membrane to acceptor phase. This is important factor to be remembered when one designs any type of carriers and some compromise has to be taken into account between selectivity and affinity [13].

3. The carrier has to be hydrophobic because it is crucial for the membrane stability and performance. The carrier molecule showing low solubility in organic phase is easily dissolved in aqueous phases. Hence, even if it is a good host for transported molecule, the entire effect of separation cannot be observed because the carrier might leak from organic membrane phase [13].

In conclusion, we have to emphasize that it is not simple and straightforward to design a good liquid membrane system for stereoisomers separation. There are some significant factors that have to be considered. This design has to be based on the knowledge of the specific interactions between host and guest molecules and guest structure. Moreover, it is necessary to pay attention to specificity of liquid membrane transport. To achieve this goal, a lot of such attempts have been made and their description is a subject of the following paragraphs.

APPLICATION OF CHIRAL LIQUID MEMBRANE PHASE

The use of chiral membrane solvent is not very widespread method in order to achieve transport stereoselectivity. In fact, there is only one work published dealing with amino acid enantiomers separation by means of chiral alcohols, nopol (*I*) and (2*S*)-(-)-methylbutan-1-ol (*II*) [14]. The presented results show that optical separation of six pairs of enantiomers of amino acids (Ala, Leu, Phe, Met, Ser, Thr, and Glu) is possible. However, the chiral discrimination (expressed as a flux ratio for both enantiomers and denoted as α) was moderate and the best result reached the value of 1.27 for serine. As a main conclusion it was stated that the factor involved in chiral discrimination was an asymmetry of amino acid molecule.



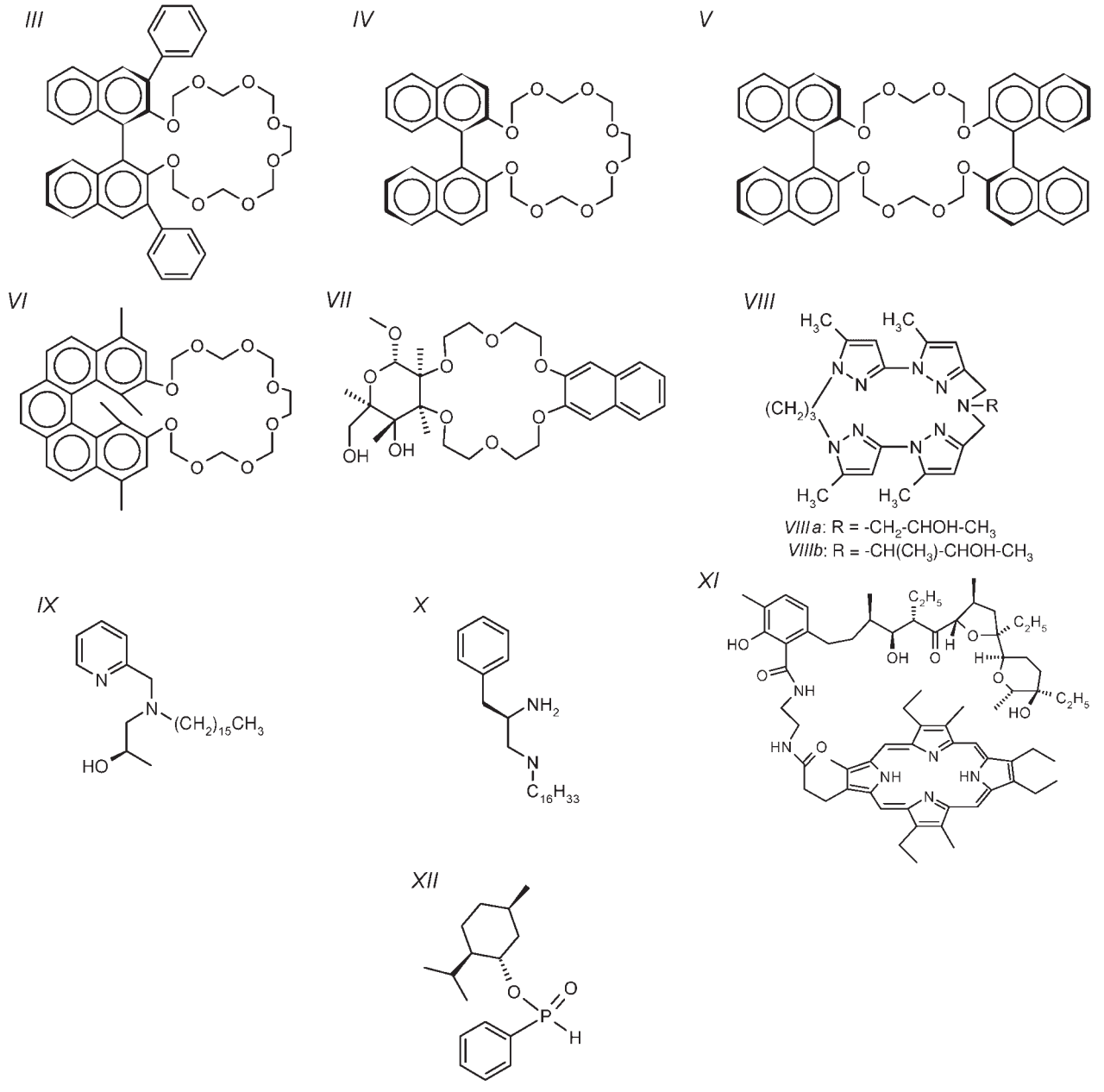
CARRIER-MEDIATED TRANSPORT

The most extensively examined way of stereoselective separation of amino acids and related compounds is carrier-facilitated transport. For convenience and clarity of this paper, the transported substances discussed herein were divided into three groups: free amino acids, amino acid derivatives, and short peptides. The main effort of discussion is to show different approaches of carrier structure design and to present some effects of structure manipulation.

Amino Acid Enantiomers

Unprotected amino acid enantiomers were transported through the liquid membranes with several types of carriers, *III*—*XII*. One of the first reports about enantiomeric resolution of amino acids by SLM [15] has described the application of chiral crown ether 2,3,4,5-bis(3-phenylnaphtho)-1,6,9,12,15,18-hexaoxacycloeicosa-2,4-diene (*III*) as the stereoselective discriminator for series of amino acids – phenylglycine (Phg), phenylalanine (Phe), tryptophan (Trp), leucine (Leu), isoleucine (Ile), *tert*-leucine (Leu), methionine (Met), valine (Val), and tyrosine (Tyr). Among these compounds the best separation factor ($\alpha = J_D/J_L$) was obtained for Phe ($\alpha = 22.7$ for racemic mixture) but also other racemic mixtures of examined amino acids were resolved with high separation factor. The interesting conclusion was that amino acids were better separated into enantiomers from their racemic

mixture than expected after comparison of particular fluxes for both antipodes separately. The explanation of this phenomenon was that the competitive transport took place in the case of racemate. For this class of carriers the co-transport mechanism was observed and the degree of separation as well as the flux values were dependent on the type of co-ion. In case of carrier *III*, its binding to amino acid occurred by hydrogen bonds between positively charged amino group and oxygen atoms of the ring. Moreover, the stereoselectivity was enhanced by the π—π interactions of aromatic part of amino acid (if it is present) or by sterical repulsion (for amino acid without aromatic subunit) and the naphthalene part of the carrier. Crown ether (*III*) has been extensively investigated due to its significant potency as a chiral discriminator [16, 17]. Based on its structure the family of similar crown ethers was synthesized (compounds *IV*—*VI*) and tested as chiral carriers [18]. It turned out that these substances are not better discriminating factors than compound *III* when phenylglycine enantiomers were transported through SLM. However, there is some discrepancy between the values of α for separation of this amino acid by this carrier mentioned in two different reports presented by the same authors [15, 18]. The separation factors equal 4.00 and 9.69 for racemic mixture of phenylglycine depending on the literature source. Therefore, those results should be taken with some reservation.



The other interesting compound from this group was reported by *Pietraszkiewicz* and coworkers [19]. It was optically active crown ether derived from D-mannose (*VII*). In BLM experiments the aromatic amino acids and their potassium and sodium salts were transported over chloroform liquid membrane containing compound *VII*. The results presented indicate that enantioselective molecular recognition lies in interactions between the naphthalene subunit and the aromatic part of amino acids. Additionally, the creation of hydrogen bonds between carboxylic group of amino acid and hydroxyl group of mannose unit was also considered. The best separation factor 3.75 was obtained for PhAlaNa (phenylalanine sodium carboxylate) and Trp (tryptophan). It was also shown that the free amino acids are bonded more strongly than their salts.

The different type of macrocyclic compounds was applied by *Boudouche et al.* [20] in CH_2Cl_2 bulk liquid membrane, where compounds *VIIIa* and *VIIIb* were used as carriers for transporting Trp and Phe lithium salt. The carrier molecule was macrocycle containing two bipyrazolic subunits and a functionalized side arm bearing one chiral carbon atom. The complexation of amino acid carboxylate took place by hydrogen bonding between nitrogen atoms of macrocycle and acidic group of amino acid. It is additionally enhanced by the interaction of side arm of bipyrazolic subunits with the guest molecule. It can be said that this class of compounds represents in fact the reversion of molecular recognition of crown ethers. In this case the chiral discrimination was not significant and percentage of enantiomer D contents of Trp and Phe was 49—51 % (α = 1.00—1.05) after the transport. It could mean that the separation factor is very close to unity and does not exceed the experimental error.

The next group of carriers is formed by species that utilize interactions between amino acids and carriers *via* transition metal complexes. The carriers for this purpose were designed and developed by *Scrimin* and coworkers [21, 22]. In this case compounds such as *IX* and *X* are able to recognize amino acid—Cu(II) complex by acting as an additional chiral ligand for copper cation. The whole complex of single enantiomer became diastereoisomeric and membrane was able to differentiate both optical isomers. The transport mechanism can be described as co-transport, in which on the interface of the organic and aqueous phases the whole amino acid—Cu(II) complex is exchanged by proton. The enantioselectivity for these compunds is rather modest and reaches the value of 2.34 for carrier *X* and Leu.

Sessler and *Andrievsky* [23] synthesized and applied sapphyrin-lasalocid conjugates (*e.g.* compound *XI*) for zwitterionic aromatic amino acids separation by BLM with CH_2Cl_2 as membrane phase. The obtained results indicate that this compound turned out to be a good chiral resolving agent towards L-enantiomers of Phe, Tyr, and Trp. The amino acid molecule in double ionic form reacts with carrier in such manner that carboxylic group is bounded by hydrogen bond with sapphyrin ring and ammonium group interacts with ether oxygen atoms. In this case the counter-current transport occurs with Na^+ cations as counter-ions. The stereoselectivity was assured by the chirality of lasalocid molecule and was in the range 1.5—2.0.

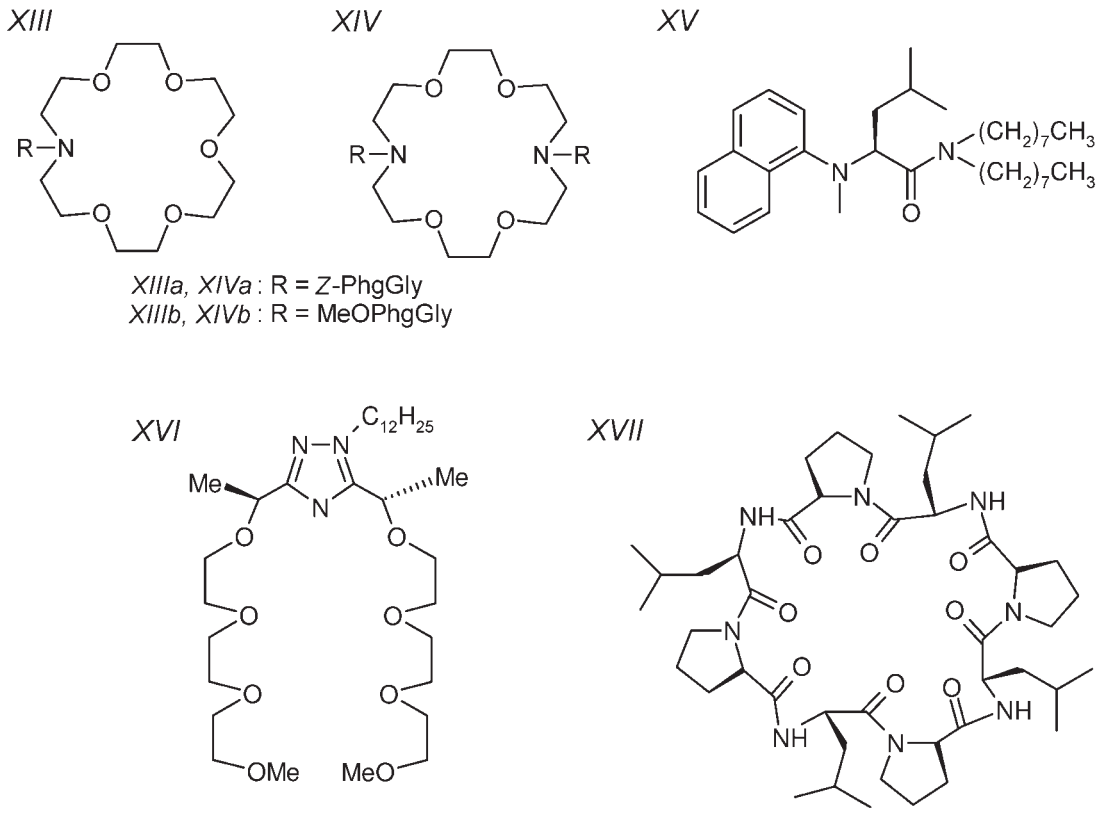
Another class of substances tested as potential chiral carriers for aromatic amino acid transport thorough SLM were dialkyl and monoalkyl phosphates, phosphites, and phosphinates based on (-)-menthol and (-)-nopol [24]. The series of nine carriers of this type were examined. They acted as anionic exchangers (able to bind cationic form of amino acid) in which the cation was bonded by phosphorus-containing group. The alcohol and aromatic subunits were responsible for chiral interactions. The amino acid was transported through membrane constituted by dihexyl ether solution of a carrier according to counter-transport mode. Protons played the role of driving force for the process. In this case the influence of carrier as well as aromatic amino acid structure on the magnitude of enantiomers transport was investigated. The obtained results show that the enantioselectivity of such carriers is moderate and for the best case (compound *XII*) reaches the value of 1.34. The interesting fact, however, concerns the fluxes values, which are higher than the fluxes obtained in case of BLM transport and therefore those carriers are more effective transporters in the sense of mass transport.

Summing up, it can be concluded that liquid membranes can separate the enantiomers of amino acids when special carriers are applied. However, with the exception of carrier *III*, the other compounds are moderate stereoselective selectors (in most cases the α value is in the range 1.5—4). Even if rather structurally fitting receptor based on sapphyrin ring and lasalocid is used still the separation factor is moderate.

Amino Acid Derivatives

N- or *C*-terminal derivatives of amino acids are the second group of compounds to be attempted for stereoselective separation in liquid membranes. Such compounds were transported mainly with the carrier-containing liquid membranes. The modes of transport are the same as in the case of free amino acids, although different carriers (*XIII*—*XVII*) were used for this purpose.

The enantioselective transport of *Z*-amino acid salts (*Z* – benzyloxycarbonyl) through BLM was examined with application of dipeptide-derived lariat ethers (*XIII*, *XIV*) [25]. Such carriers possess the ability of structural and chiral recognition of *Z*-PheO$^-$ and *Z*-PhgO$^-$ anions. They do that by the simul-



taneous complexation of K^+ and carboxylate part of amino acid by lariat molecule strengthened with hydrogen bonds. The dipeptide arm of conjugate enhances the enantioselective recognition. Such complex structure caused that the transport mechanism was of co-transport mode. The average separation enantioselectivity was estimated at 1.5 and took the best value for compound *XIVb* ($\alpha = 1.7$).

The other type of *N*-blocked derivative, DNB-Leu (DNB – 3,5-dinitrobenzoyl) was successfully separated from the racemic mixture by using chiral surfactant in hollow-fibre liquid membrane contractor [26]. From the chemical point of view the carrier molecule was the amide of (*S*)-*N*-(1-naphthyl) leucine and fatty acid (compound *XV*). In this case the binding of DNB-Leu proceeds by the creation of hydrogen bonds between amino acid part of the interacting molecules. Stereoselective recognition was strengthened by π—π aromatic rings stacking. The mechanism type presented in this case is diffusion-enhanced by the presence of carrier. This liquid membrane system enabled to purify preparatively (with 95 % of enantiomeric excess, $\alpha = 20$) one of the enantiomers and can be example of very practical application of liquid membrane for stereoselective separation.

Chiral amino acid esters were also attempted to separation by liquid membrane carrier-mediated transport. 1,2,4-Triazole-containing podands and macrocycles (for example compound *XVI*) [27] were the class of carriers acting as chiral discriminators in BLM for methyl PhGly. The discrimination is moderate, as authors concluded, and is about 20 % of enantiomeric excess ($\alpha = 1.5$). The transport mechanism can be described as a co-transport with ClO_4^- as a co-ion. The carrier molecule binds to amino acid ester salt in the same manner as crown ethers and the chiral discrimination is caused by the presence of chiral centres (steric interactions) in proximity of triazole aromatic ring.

Macrocyclic pseudopeptides containing *N*,*N*′-ethylene-bridged dipeptide units (*e.g.* compound *XVII*) designed by *Miyake* and coworkers [28] are very interesting carriers for amino acid esters salts separation. The authors found them to be effective transporters and chiral selectors for amino acids methyl esters. They concluded that the transport mechanism was similar to crown ethers-mediated separation. The enantiomeric separation varied from 3 % to 30 % of enantiomeric excess (α varied from 1.0 to 2.10) and depends on the type of applied macrocycle and ester structure. The best resolution was obtained for $TyrOMeH^+$ (36 % e.e. towards enantiomer *S*). The

chiral recognition is again caused by the sterical repulsion between amino acids side chain and the groups located around chiral centres present in macrocycle.

Summarizing this part of the review, it can be said that with one exception [26] the separation factors are of the same values as for free amino acids. However, in this case another types of molecules were examined as carriers for enantioselective separation.

Short Peptides

There are few reports about the enantioselective separation of peptides realized by means of liquid membranes. In one of the first papers, *Kafarski et al.* [29] described separation of diastereoisomers AlaAla and AlaAla phosphono analogue with achiral crown ethers as transport enhancers. The obtained results show that D,L isomers are transported faster than their L,L forms. The results of this work were further applied to other dipeptides and phosphono dipeptides [30]. Transport of nine L,D or L,L diastereoisomers was examined and as a conclusion the authors assume that L,L dipeptides are transported with the higher rates than L,D isomers and the separation factor varies from 0.7 for ProAla-phosphono analogue to 2.2 for AlaAla. Moreover, the stereoselectivity is independent of the presence on carrier, in this case achiral crown ethers, but the application of achiral crown ethers significantly increases the transport rate of both diastereoisomers.

Interesting separation of dipeptides enantiomers was achieved by *Žinić et al.* [25]. They applied dipeptide-derived lariat ethers (*XIVa* and *XIVb*) and studied the separation of *Z*-L,D-PhgGlyOK and *Z*-Gly-L,D-PhgOK. The studies showed that it was possible to separate enantiomers but the stereoselectivity was as low as 1.5.

At the end of this part it is noteworthy to conclude that the stereoselective separation of short peptides should proceed with higher degree for diastereoisomers than amino acid or peptide enantiomers due to differences in physicochemical properties.

CONCLUSION

The presented examples for stereoselective transport of amino acid related compounds show that several approaches for such phenomenon can be proposed. The separation can proceed by the simple application of chiral organic phase or by designing of carriers with their complicated structures of molecules that act as "real" receptors for amino acid enantiomers. The different types of transport mechanism can also be distinguished. The most popular one seems to be co-transport mode. The popularity comes from the fact that most frequently used carriers are based on crown ethers structure.

The stereoselectivities obtained by application of carrier-mediated liquid membrane separation are very different and depend on the structure of guest and host molecules. The magnitudes of the stereoselectivity are in most cases moderate but similar to other membrane-based stereoisomers separation techniques. However, the advantage of application of liquid membrane systems is the significantly higher fluxes that is a result of higher diffusion rates through liquid medium. The only problem that can be encountered is the liquid membrane stability. Deterioration of the membrane performance is caused by liquid membrane phase and/or carrier leakage. To overcome this obstacle the careful selection of process conditions and designing of new chiral carriers with special attention paid to carrier hydrophobicity should be performed. It is still possible to look for the new carriers with the better ability to stereoselectively recognize amino acids or peptides stereoisomers.

These facts cause that liquid membranes can be considered as an effective method for stereoselective separation of amino acid-derived compounds. They may also serve as a valuable tool for the study of physicochemical mechanisms of separation and other supramolecular processes in terms of specific interactions between host and guest amino acid or peptide molecules. Therefore, the efforts and search for new mechanisms of realization of enantioselective transport are still promising.

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