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Permeability of membranes to amino acids and modified amino acids: Mechanisms involved in translocation

Review Article

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Summary. The amino acid permeability of membranes is of interest because they are one of the key solutes involved in cell function. Membrane permeability coefficients (P) for amino acid classes, including neutral, polar, hydrophobic, and charged species, have been measured and compared using a variety of techniques. Decreasing lipid chain length increased permeability slightly (5-fold), while variations in pH had only minor effects on the permeability coefficients of the amino acids tested in liposomes. Increasing the membrane surface charge increased the permeability of amino acids of the opposite charge, while increasing the cholesterol content decreased membrane permeability. The permeability coefficients for most amino acids tested were surprisingly similar to those previously measured for monovalent cations such as sodium and potassium (approximately 10^{-12}–10^{-13} cm · s^{-1}). This observation suggests that the permeation rates for the neutral, polar and charged amino acids are controlled by bilayer fluctuations and transient defects, rather than partition coefficients and Born energy barriers. Hydrophobic amino acids were 10^2 more permeable than the hydrophilic forms, reflecting their increased partition coefficient values.

External pH had dramatic effects on the permeation rates for the modified amino acid lysine methyl ester in response to transmembrane pH gradients. It was established that lysine methyl ester and other modified short peptides permeate rapidly ($P = 10^{-2}$ cm · s^{-1}) as neutral (deprotonated) molecules. It was also shown that charge distributions dramatically alter permeation rates for modified di-peptides. These results may relate to the movement of peptides through membranes during protein translocation and to the origin of cellular membrane transport on the early Earth.

Keywords: Amino acid – Modified amino acid – Permeability – Liposome – Membrane

Abbreviations: DCP, dicetylphosphate; DMPC, dimyristoyl phosphatidylcholine; EPC, egg phosphatidylcholine; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; PLM, planar lipid membrane; SUV, small unilamellar vesicle; ΔpH, transmembrane pH gradient

Introduction

Lipid bilayers are generally impermeable to most polar or charged molecules. However, cells must be able to transport nutrients and regulate intracellular ion concentrations. Specialized membrane transport proteins evolved to allow for the transport of specific ionic solutes and families of molecules, including sugars and amino acids. These transport proteins have been extensively discussed elsewhere (see Gunn, 1980 for a review) and will therefore not be dealt with in this article. However, the permeability properties of the lipid bilayer itself are of interest for establishing the "background" levels of transport allowed by the membrane. Such information also provides a perspective on solute permeation in natural situations prior to the evolution of transport proteins or where transport proteins are absent.

The general barrier properties of the bilayer have been well-established by previous studies, particularly with respect to ionic solutes (sodium, potassium, chloride, hydrogen ion), polar solutes (water, glucose) and small neutral molecules (glycerol, urea) (see Deamer and Bramhall, 1986 for an excellent review). However very little work has been done on more complex ionic solutes such as the amino acids, clearly one of the most important types of molecules involved in cell function and replication.

In this article, I will describe the current views on how amino acids and modified amino acids permeate lipid bilayers using direct and indirect permeability measurements in model membrane systems. Initially, I will discuss the model systems of biological membranes employed for these types of studies. I will then describe how amino acid permeability is measured and what factors can influence it. The work that has been done regarding the permeability of modified amino acids will then be presented. Finally, I will comment on the biological relevance of the different mechanisms that have been proposed to account for the permeabilities of amino acids and modified amino acids.

1. What model systems are most suitable for measuring amino acid permeabilities?

A. Planar lipid membranes

Planar lipid membranes (PLM) were one of the earliest model membrane systems used for permeability measurements (Montal and Mueller, 1972). PLMs are created by applying a mixture of lipid in organic solvent to an aperture separating two aqueous compartments (Cullis and Hope, 1985). The solvent collects at the perimeter of the aperture, creating a lipid bilayer across it. The major advantages of planar membranes are that electrodes can be easily introduced, solutions can be altered quickly and, thus, sensitive measurements of ion gradients can be achieved (Fettiplace et al., 1974). These bilayers have proven

useful for studying pores, channels and transporters. The major disadvantages of this technique include the small amount of lipid present, permeability changes caused by residual organic solvent and possible artifacts at the lipid-aperture interface (Cullis and Hope, 1985). These disadvantages are directly relevant to the measurement of amino acid/modified amino acid permeability and therefore indicate that other model systems are preferable.

B. Liposomes

Liposomes are the model system of choice for the type of permeability measurements described in this work. The term "liposome" is used to describe an aqueous dispersion of bilayer forming lipids. The three major types of liposomes are shown in Fig. 1 (Cullis et al., 1989). Liposomes were first created by simply dispersing bilayer forming lipids in water (Bangham et al., 1965). Liposomes have several attributes that make them particularly suitable as model membrane systems for determining amino acid permeability. First, liposomes have a very large membrane surface area (six orders of magnitude larger than a typical PLM; Deamer and Bramhall, 1986). This results in a large surface area:volume ratio, which increases the likelihood of observing infrequent membrane permeation events. The stability of liposomes allows for the observation of slow permeation processes. Liposomes can also be used *in situ* with a variety of instruments, such

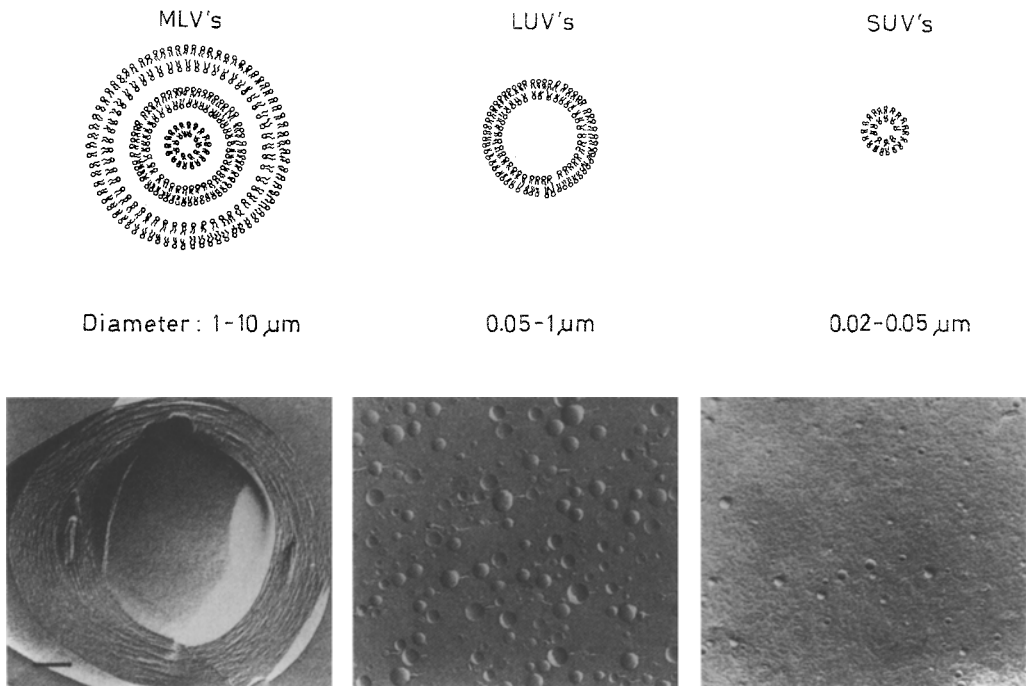

Fig. 1. Multilamellar vesicles (*MLVs*), large unilamellar vesicles (*LUVs*) and small unilamellar vesicles (*SUVs*) in diagrammatic form and as visualized by freeze-fracture electron microscopy. All liposomes were composed of egg phosphatidylcholine. The scale bar represents 200 nm (reproduced with permission from Cullis et al., 1989)

as fluorimeters, spectrophotometers and magnetic resonance spectrometers because they exist as a fluid suspension and do not scatter light effectively at most wavelengths (Deamer and Bramhall, 1986).

Liposomes may be small (≤ 50 nm diameter) or large (>50 nm diameter) and may consist of many concentric bilayers or a single lipid bilayer, depending on the technique used. For example, multilamellar vesicles (MLVs) can be created by simply dispersing lipid in buffer (see Fig. 1). However MLVs are of limited value for permeability studies due to their multilamellar nature and heterogeneous size distribution. The best liposome systems for permeability measurements are the Small and Large Unilamellar Vesicles (SUVs and LUVs; see Fig. 1). These are the preferred systems because all permeation events occur through only a single bilayer encapsulating an aqueous volume of defined size (Cullis and Hope, 1985). Most of the permeability work that will be discussed in this paper was done with SUV and LUV systems.

Sonication of lipid dispersions (Huang, 1969) or passage through a French press (Barenholtz et al., 1979) results in the creation of SUVs (25–50 nm diameter). The principal advantages of SUV systems are that they are easy to prepare and are relatively homogeneous in size. The major disadvantages to SUVs are that they are unstable (tend to fuse), have small trapped volumes (<0.2 μl/μmole phospholipid) and have the potential for generating lipid oxidation products during preparation (Cullis and Hope, 1985). In addition, permeability coefficients for ion transport across SUV membranes are typically 1–2 orders of magnitude smaller than for the corresponding LUV systems (Perkins and Cafiso, 1986). The explanation for this anomalous behavior is unknown. These disadvantages indicate that SUVs may not be ideal for many types of permeability measurements.

LUVs overcome all the disadvantages of SUVs and have therefore become the main model membrane system employed for permeability measurements. LUV systems may be produced using a variety of techniques, including the use of detergents (Mimms et al., 1981) or organic solvents (Szoka and Papahadjopoulos, 1980; see Hope et al., 1986 and Hope et al., 1992 for excellent reviews on this subject). The technique of choice for production of LUVs involves the repeated passage of MLVs through filters of defined pore size under medium pressure. This procedure originated from the initial observation by Olson et al. (1979) that greater size homogeneity could be obtained for vesicles following low pressure extrusion through polycarbonate filters of a defined pore size. The technique was further developed by Cullis and co-workers (Hope et al., 1985) and is now in widespread use. The main advantages of this technique include its speed, the avoidance of solvents/detergents, the high trapping efficiencies obtained and the homogeneous nature of the resulting vesicle populations (Hope et al., 1986). The only minor disadvantage of this procedure is that homogeneous unilamellar vesicle populations of sizes larger than 200 nm cannot be created. Larger vesicles can be created but as filter pore size increases, an increasing proportion of the vesicles will be multilamellar and the vesicle populations will therefore become more heterogeneous. Thus, LUVs produced using the 100 or 200 nm filter pore sizes are the best model membrane systems for most permeability measurements.

2. How do amino acids permeate lipid bilayers?

The current understanding of membrane permeability is based upon the principle that lipid bilayers serve as the major barriers to the free diffusion of water and solutes. There are two primary models that have been suggested to explain the permeability properties of lipid bilayers. These two models, the transient defect and solubility-diffusion models, are both strongly supported by experimental evidence and almost certainly play roles in the permeation of amino acids and modified amino acids through membranes. Both of these mechanisms will therefore be briefly explained and will be expanded upon in subsequent sections of this paper.

A. Transient defects

The energy requirements for the movement of an ion from a high dielectric medium (water) to a low one (membrane) are quite significant (approximately 40 kcal/mole for a monovalent ion; Parsegian, 1969; Deamer and Bramhall, 1986). Transient defects were first described by Nagle and Scott (1978) as cavities which allow small ions to enter and subsequently pass through the bilayer without having to overcome the Born energy required for a charged molecule to "dissolve" in the low dielectric membrane interior (Parsegian, 1969). In general, maximal ion flux occurs at the T_c (the gel to liquid-crystalline phase transition temperature) of the membrane. At this temperature the gel and liquid-crystalline phases co-exist, resulting in 'lateral phase separation'. This means that in areas where part of the membrane is gel state and part is fluid, there may exist regions where lipid molecules are alternating between the two states or are in some intermediate form. In either case, this will result in an area of membrane instability (producing transient defects) which will reduce the activation energies for ion permeation (Deamer and Bramhall, 1986).

B. Solubility-diffusion (translocation of neutral species)

This model assumes that molecules dissolve in the non-polar core of the bilayer, cross via simple diffusion and that membrane barriers to entry and exit are insignificant (Deamer and Bramhall, 1986). The specific example of membrane transport employing solubility-diffusion that is relevant to this work involves the translocation of weak acids and bases in response to ΔpH. Such transport has been previously demonstrated for a variety of molecules in model membrane systems (see Deamer et al., 1972; Nichols and Deamer, 1976; Madden et al., 1990; Cullis et al., 1991 and Chakrabarti et al., 1992 for additional details). This phenomenon arises from rapid transbilayer movement of the neutral form of the weak acid or base, which can result in large transbilayer concentration gradients. For example, a ΔpH (acidic interior) serves to produce a considerable inward net transport of weak bases by trapping the neutral membrane-permeable species in the charged (protonated) form after they traverse the bilayer. Hence, the weak base accumulates in the acidic compartment of the liposome. Raising the external pH will increase the proportion of exterior molecules in the neutral form

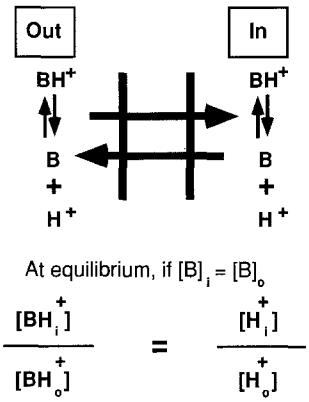

Fig. 2. Redistribution of a weak base in response to ΔpH, where *B* represents the neutral form of the weak base of interest

and hence the rate and extent of transport. It is straightforward to show that, in the absence of membrane partitioning effects, lipophilic amines will be accumulated into LUVs with an acidic interior to achieve inside/outside concentration ratios which correspond to the inside/outside concentration ratios of protons (see Fig. 2). A ΔpH of three units, for example, can result in interior concentrations of weak bases which are 1000 times larger than exterior values.

3. How is membrane permeability measured?

A. Amino acids

Amino acid permeability measurements can be obtained using a variety of techniques such as electron spin resonance (Cantenys et al., 1983), radiolabeled amino acids in conjunction with planar lipid membranes (Stillwell and Winter, 1973) and amino acid oxidases (Naoi et al., 1977). An easy and more generally applicable technique involves the passive entrapment of amino acids in liposomes, immediately followed by gel filtration to remove any free amino acid present (Sada et al., 1990; Chakrabarti and Deamer, 1992). The efflux of the entrapped amino acid from the liposomes can then be measured via the use of fluorescamine or other probes. Fluorescamine is particularly suitable for this type of work because of its sensitivity (nmole amounts of amino acid) and because it reacts with primary amino groups (Udenfriend et al., 1972).

The kinetics for the efflux of passively entrapped amino acids uses an initial rate analysis, which assumes that the concentration of amino acid inside the liposome is much greater than that on the outside. The efflux process should obey the relation

$$A(t)_{ex} = A(eq)_{ex}(1 - e^{-kt})$$

where $A(t)_{ex}$ is the exterior concentration of amino acid at time t, $A(eq)_{ex}$ is the equilibrium exterior concentration at t = infinity and k is the rate constant associated with the efflux process (Cullis et al., 1993, manuscript in preparation).

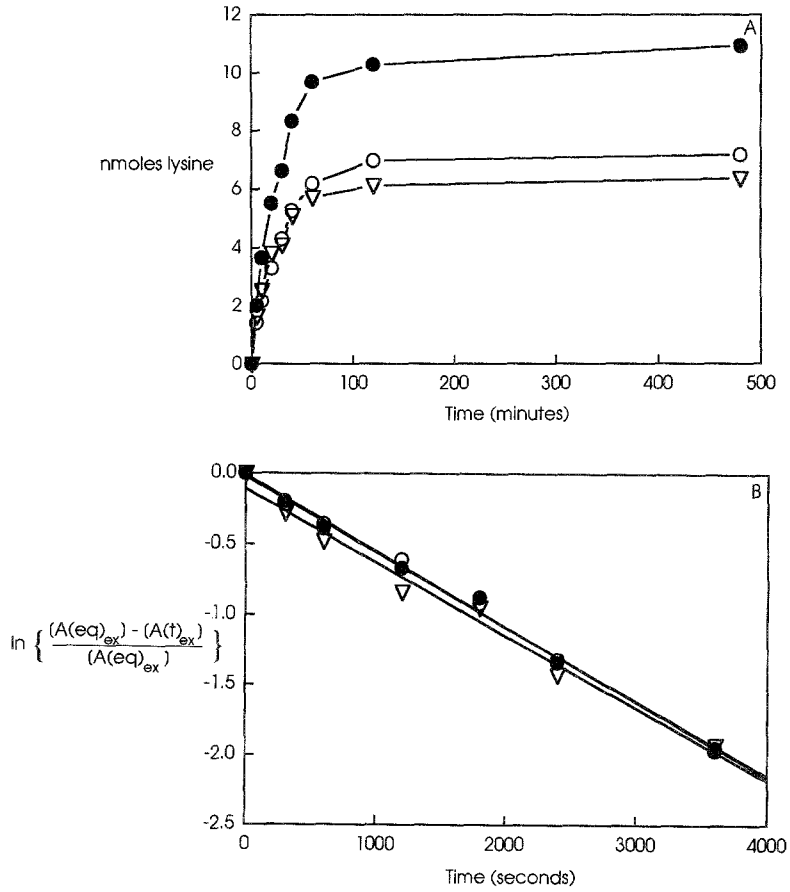

Fig. 3. A Time course of release of lysine from 200 nm DMPC vesicles at various pH values. The pH values of the samples were 4.0 (o), 6.0 (•) and 8.0 (▽). The experiment was conducted at 31°C and the initial lysine concentration was 50 mM. **B** Plot of $\ln\{([A(eq)]_{ex} - [A(t)]_{ex})/[A(eq)]_{ex}\}$ versus time.; where $[A(eq)]_{ex}$ is the exterior concentration of amino acid at equilibrium and $[A(t)]_{ex}$ is the exterior concentration of amino acid at time t. The slopes of the lines gives the rate constant (k) for the transbilayer diffusion of lysine (modified from Chakrabarti and Deamer, 1992)

Thus, a plot of $\ln\{([A(eq)]_{ex} - [A(t)]_{ex})/A[(eq)]_{ex}\}$ versus t should give a slope of $-k$, the rate constant. Rate constants can be determined by applying a non-linear least-square analysis to efflux data. An example of this type of derivation for lysine efflux from 200 nm DMPC liposomes is shown in Fig. 3.

Permeability coefficients (P) can then be calculated using the expression

$$P = (V_0/A_m)k$$

where P is expressed in $\text{cm} \cdot \text{s}^{-1}$, k is the rate constant, V_0 is the aqueous volume of the lipid dispersion and A_m is the area of the LUV membrane. This expression can be readily simplified to:

$$P = (r/3)k$$

where r is the radius of the vesicle (Chakrabarti and Deamer, 1992).

B. Modified amino acids

The uptake of a modified amino acid, lysine methyl ester, into liposomes in response to ΔpH was studied to determine its membrane permeability (Chakrabarti et al., 1992). The kinetics of the uptake process were analyzed assuming

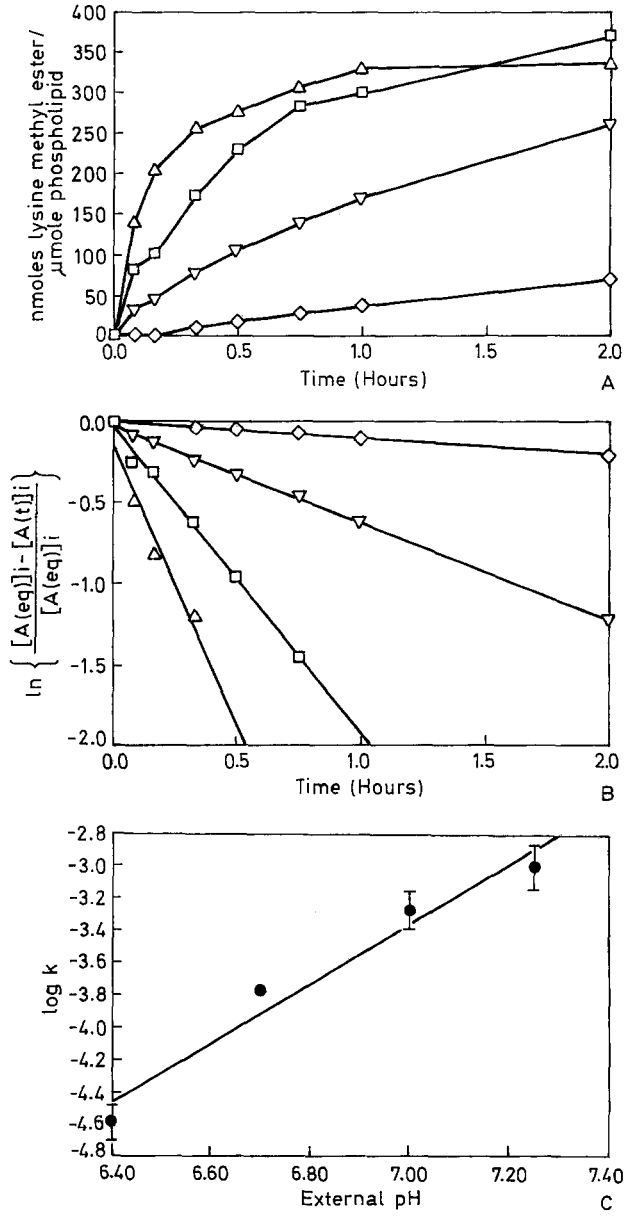

Fig. 4. A Effect of external pH on the uptake of lysine methyl ester into 100 nm EPC vesicles in response to ΔpH. The exterior/interior pH values of the samples were 6.4/2.9 (◇), 6.7/3.2 (▽), 7.0/3.5 (□) and 7.25/3.75 (△). **B** Plot of $\ln\{([A(eq)]_i - [A(t)]_i)/[A(eq)]_i\}$ versus time.; where $[A(eq)]_i$ is the interior concentration of amino acid at equilibrium and $[A(t)]_i$ is the interior concentration of amino acid at time t. The slopes of the lines give the rate constant (k) for the transbilayer transport of lysine methyl ester. **C** Plot of log k vs. external proton concentration. The slope of this line is 1.85 (from Chakrabarti et al., 1992)

that only the neutral (deprotonated) form of the amino acid could move across the lipid bilayer. As developed more completely elsewhere (Cullis et al., 1993, manuscript in preparation), the accumulation process should then obey the relation

$$[A(t)]_i = [A(eq)]_i(1 - e^{-kt})$$

where $[A(t)]_i$ is the interior concentration of the amino acid or peptide at time t, $[A(eq)]_i$ is the equilibrium interior concentration at t = infinity and k is the rate constant associated with the uptake process (Chakrabarti et al., 1992).

Lysine methyl ester contains two ionizable (primary) amino functions with corresponding dissociation constants K_1 and K_2. Under the assumption that only the neutral (fully deprotonated) form is able to translocate the membrane, it is readily shown that

$$k = (P\ A_m/V_0)(1 + [H^+]_0/K_1 + [H^+]_0{}^2/K_1K_2)^{-1}$$

where P is the permeability coefficient for the neutral form, A_m is the area of the LUV membrane, V_0 is the aqueous volume of the lipid dispersion and $[H^+]_0$ is the exterior proton concentration. Thus if $K_1, K_2 \ll [H^+]_0$, we obtain

$$k = P\ A_m\ K_1K_2/V_0[H^+]_0{}^2$$

It should be noted that P is an effective permeability coefficient which is related to the actual membrane permeability coefficient, P_m, by the relation $P_m = K\ P$ where K is the membrane-water partition coefficient of the charged (protonated) species of the amine. Also, this is an initial rate analysis and assumes that the concentration of the neutral amine on the outer surface of the LUVs is much greater than that on the inner surface. The rate constant, k, can be calculated from the slope of a plot of ln $\{([A(eq)]_i - [A(t)]_i)/[A(eq)]_i\}$ versus t, for the lysine methyl ester. The rate constant (k) can be determined by applying a non-linear least-square analysis to the uptake data using a commercially available plotting program, employing k and $[A(eq)]_i$ as variables (Chakrabarti et al., 1992). Figure 4 shows an example of how rate constants can be derived from the translocation of lysine methyl ester in response to ΔpH.

4. What factors influence amino acid permeability?

A. Effect of amino acid structure

Early work in this area by Klein et al. (1971) found that amino acid structure influenced permeability, with hydrophobic amino acids being more permeable than charged forms. They also found that increasing the degree of unsaturation of membranes increased their permeability to amino acids. This pioneering work established a basic order of amino acid permeability (phenylalanine > methionine > leucine > isoleucine, norleucine > alanine, glycine > histidine) but was limited by the techniques then available for production of liposomes. The vesicles employed were of variable size and were multilamellar in nature; hence the surface areas available for diffusion were unknown (Klein et al., 1971). These uncertainties made precise permeability coefficient calculations impossible.

Table 1. Average permeability coefficients for various classes of amino acids using 200 nm EPC and DMPC vesicles (modified from Chakrabarti and Deamer, 1992)

Amino acid	P (EPC; 10^{-11} cm·s^{-1})	P (DMPC; 10^{-11} cm·s^{-1})
Glycine	0.57	2.0
Serine	0.55	1.6
Lysine	0.51	1.9
Tryptophan	41	—
Phenylalanine	25	—

Technical developments since 1971 in the preparation of liposomes have allowed for more precise measurements of amino acid permeation to be made. Table 1 gives the average permeability coefficients that have been recently determined for examples from the various classes of amino acids in EPC and DMPC vesicles (Chakrabarti and Deamer, 1992). The order of amino acid permeability (tryptophan, phenylalanine $\gg$ glycine, serine, lysine) is similar to that observed by Klein et al. (1971). Naoi et al. (1977) also established the following order of amino acid permeability: leucine > phenylalanine > tryptophan > methionine > tyrosine, valine > threonine > serine > alanine > glycine. This corresponds quite well to the previous permeability orders, except for leucine and phenylalanine which are reversed in order when compared to Klein et al. (1971).

The similarities in permeability coefficients for the neutral, polar and charged amino acids, together with the observation that transport is unaffected by pH (see Section 4.B.), indicates that a mechanism other than translocation of the neutral species is likely responsible for the observed efflux (Chakrabarti and Deamer, 1992). It seems probable that the efflux of amino acids occurs because of transient defects or "leaks" in the membrane (Nagle and Scott, 1978). Naoi et al. (1977) observed that bilayers were approximately 25–30 times more permeable to hydrophobic amino acids than charged amino acids such as lysine. However they used the indirect determination of amino acid flux through measurement of oxidation rates via the use of passively entrapped D-amino acid oxidase (Naoi et al., 1977). Chakrabarti and Deamer (1992) used a more direct approach which found that bilayer permeability to hydrophobic amino acids may be up to 100 times higher when compared with other amino acids (Table 1).

The permeability differences between the hydrophobic and other amino acids appears related to differences in their partition coefficients between the organic and water phases (Chakrabarti and Deamer, 1992). Klein et al. (1971) also found a correlation between the water : octanol partition coefficients for the amino acids they studied and the rates of efflux measured. Table 2 gives previously published partition coefficient data for most of the amino acids that were examined by Chakrabarti and Deamer (1992; Leo et al., 1971). Both phenylalanine and tryptophan partition into the organic phase (roughly equivalent to the lipid bilayer) 10–100 times more readily than any of the aliphatic or charged amino acids. It follows that the translocation of hydrophobic amino acids may

Table 2. Partition coefficients for some of the amino acids studied (from Leo et al., 1971)

Amino acid	Log *P* octanol
Tryptophan	−1.04
Phenylalanine	−1.43
Lysine	−2.82
Glycine	−2.26 (average)

involve a modest amount of partitioning into the membrane interior, as well as translocation through transient defects. For comparison, a truly neutral solute such as lysine methyl ester translocates entirely as a neutral entity, and can therefore permeate at rates up to 10^8 faster than the hydrophobic amino acids (Chakrabarti et al., 1992). The latter must bring charged groups into the hydrophobic phase and therefore still faces a formidable Born energy barrier.

Another possible explanation for the permeability coefficients presented here is that the neutral form of the amino acids is translocating across the bilayer. Only a very small proportion of the amino acids present would be in the neutral form (see Fig. 5 for details). The small size of the neutral population (theoretically 10^6–10^8-fold less when compared to the charged forms of an average amino acid) could account for the much slower permeation rates observed when compared to modified amino acids, such as lysine methyl ester (which are predominantly in the neutral form at basic pH values; see Section 5.A. and Chakrabarti et al., 1992).

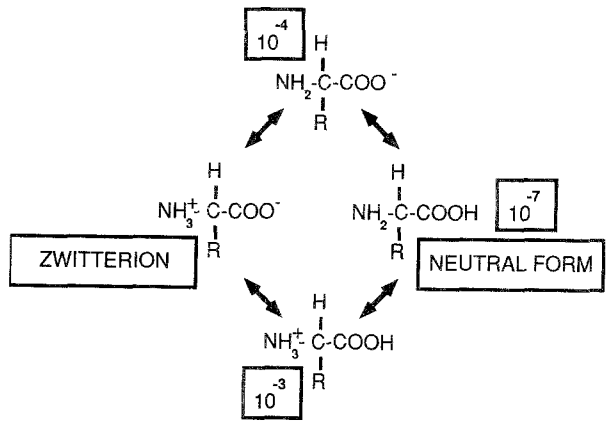

Fig. 5. Equilibrium between the zwitterionic, charged and neutral forms of a typical amino acid, where *R* represents the side-chain. Assuming that $pKa_1 = 3$, $pKa_2 = 10$ and that the pH = 7, the proportion of the population in each form can be estimated using the Henderson-Hasselbalch equation. The ratio of R-COOH/R-COO^- is 10^{-3}, while that of R-NH_2/R-NH_3^+ is 10^{-4}. Hence, the size of the neutral population is approximately 10^{-7} ($10^{-3} \times 10^{-4}$; NH_2-R-COOH/NH_3^+R-COO^-; modified from Segel, 1976)

B. Effects of pH and lipid chain length

It has been established by Chakrabarti and Deamer (1992) that amino acid permeation of lipid bilayers in LUV systems is independent of the pH (see Table 3 and Fig. 3). The length of the lipid chain did have a modest effect on permeability, with shorter chain lipids (DMPC) being slightly more permeable than longer chains (EPC) as might be expected (Table 3). Earlier research by Naoi et al. (1977) concluded that lipid composition modulated amino acid permeability. However, rather than comparing pure lipid systems of different chain lengths, that work used a variety of lipids mixed with egg phosphatidylcholine. Therefore longer chain lipids were always present and it was difficult to draw conclusions about the effect of varying hydrocarbon chain length on amino acid permeability. Results from Chakrabarti and Deamer (1992) indicate that decreasing hydrocarbon chain length from 18 (EPC) to 14 (DMPC) carbons increased amino acid permeation rates by approximately 5-fold. This increase in permeability may also be attributed to differences in chain kinking between the EPC and DMPC vesicles (presence of one or more double bonds in the EPC vesicles) and the heterogeneity in chain length of the EPC vesicles.

C. Effects of surface charge and cholesterol content

Sada et al. (1990) studied the permeabilities of several amino acids using SUVs composed of DMPC and dicetylphosphate (DCP). The permeability coefficients they determined were on the order of 10^{-10}–10^{-11} cm·s^{-1}. They found that increasing the amount of negative charge (DCP) present in the membrane enhanced the permeability of positively charged amino acids, such as lysine, but had no effect on the permeability of other amino acids. The negative charge would serve to increase the localized concentrations of lysine near the membrane, resulting in increased permeability. Both Sada et al. (1990) and Naoi et al. (1977) found that increasing the concentration of cholesterol present in the membrane decreased the permeability of amino acids. Increasing the cholesterol content of the membrane would decrease the bilayer fluidity above T_c, posing an extra barrier to permeation. In addition, increasing the concentration of cholesterol may also act to displace solute, which could otherwise intercalate with the hydrocarbon chains (Sada et al., 1990).

Table 3. Permeability coefficients (P) for the efflux of glycine from 200 nm EPC and 200 nm DMPC vesicles at various pH values (modified from Chakrabarti and Deamer, 1992)

pH	P (EPC; 10^{-11} cm·s^{-1})	P (DMPC; 10^{-11} cm·s^{-1})
3	0.73	2.6
4	0.83	1.9
5	0.40	—
6	0.50	1.6
7	0.53	—
8	0.50	1.7
9	0.47	—

D. Effects of temperature

Naoi et al. (1977) observed that temperature had a critical effect on membrane permeability to amino acids, with permeability increasing as the temperature increased. Sada et al. (1990) found that permeabilities of amino acids were extremely low below the T_c of the lipid. It is believed that these permeability differences occur because the membrane molecules are in a tightly packed gel state at temperatures below the T_c, retarding efflux. This has been noted previously for the permeation of a variety of ions, all with maximum permeability at the T_c; supporting the idea that transient defect formation may be responsible for amino acid permeability (Deamer and Bramhall, 1986). This conclusion is supported by the fact that the permeability coefficients for monovalent cations such as Na^+ and K^+ are in the range of 10^{-12}–10^{-13} cm·s^{-1}, very similar to those values measured for most amino acids (Chakrabarti and Deamer, 1992). Transient hydrated defects have been implicated as the mechanism by which these monovalent cations permeate lipid bilayers (Nagle and Scott, 1978; Deamer and Bramhall, 1986).

5. What factors influence the permeability of modified amino acids?

A. Effects of pH

Research by Chakrabarti et al. (1992) measured the permeation rates of certain modified amino acids and peptides in response to ΔpH. Lysine methyl ester was used, because it would be neutral at higher pH values and positively charged at lower pH values. The effect of ionic charge on permeation rates could therefore be determined. Dramatic pH effects were observed for the translocation of lysine methyl ester, especially when compared to unaltered lysine (see Figs. 3 and 4). It was demonstrated that it was the neutral form of the modified amino acid and peptide that was translocated across the bilayer. This translocation was very rapid ($P = 2.1 \times 10^{-2}$ cm·s^{-1}) for the lysine methyl ester (Chakrabarti et al., 1992). In subsequent work, the transbilayer movement for the unaltered amino acid lysine was determined to be many orders of magnitude slower (average $P = 5.1 \times 10^{-12}$ cm·s^{-1}) than that of the lysine methyl ester (Chakrabarti and Deamer, 1992; Chakrabarti et al., 1992). This would be expected for a zwitterion, which would remain predominantly in the charged form regardless of pH. Previous research has established that the presence of charged groups restricts transbilayer diffusion, when compared to the neutral form (Gutknecht and Walter, 1981; Chakrabarti et al., 1992).

B. Effects of charge distribution on permeability

The ability of small di-peptides, composed exclusively of basic (lysine) and hydrophobic (tryptophan) amino acids, to accumulate into LUV systems in response to ΔpH was recently investigated by Chakrabarti et al. (1993). In the case of the di-peptides Trp-Lys-amide and Lys-Trp-amide, remarkable differences in the rate constants associated with net transport were observed (Chakrabarti et al., 1993). In EPC : cholesterol (55 : 45; mol : mol) LUV systems ex-

hibiting a ΔpH of 3 units ($pH_i = 4.0$; $pH_0 = 7.0$), for example, the rate constant for the uptake of the Lys-Trp-amide is some 5×10^3 faster than for the Trp-Lys-amide. The reasons for the very large differences in the rate constants associated with uptake of the Lys-Trp-amide as compared to the Trp-Lys-amide are not clear. The partition coefficients for both molecules are comparable. Similarly, no significant difference between the pK's of the two amino groups associated with these molecules could be observed. A remaining possibility is that while the dissociation constants and abilities to partition into the lipid-water interface are similar, the ability of these molecules to enter the bilayer hydrocarbon are markedly different. Such differences, which could be related to conformational factors and/or hydrogen bonding considerations, may be reflected by the lower activation energy (23.7 kcal/mol) associated with the more rapidly translocated Lys-Trp-amide compared to the Trp-Lys-amide (29 kcal/mol) in the EPC : cholesterol (55 : 45; mol : mol) system (Chakrabarti et al., 1993).

Related effects were observed for tri-peptides composed of one lysine and two tryptophan residues, however the differences in rate constants were less sensitive to amino acid sequence. It was concluded that different charge distributions in short peptides of identical amino acid composition can strongly influence the ability of these groups to associate with and permeate across lipid bilayers (Chakrabarti et al., 1993).

C. Activation energies for the translocation of modified amino acids and peptides

Activation energies associated with the transfer of molecules from aqueous media into the membrane have been estimated according to the number of hydrogen bonds that must be broken to enter the membrane less the number created once inside (Stein, 1967; Walter and Gutknecht; 1986). Calculation of activation energies in this manner becomes more difficult as the size of the molecule increases, given the problem of estimating formation of intramolecular hydrogen bonds once inside the membrane. Previous work has shown that lysine methyl ester exhibits a high activation energy, in the range of 36 kcal/mole (Chakrabarti et al., 1992). Addition of a tryptophan residue to the lysine molecule appears to lower the activation energy by at least 9 kcal/mole (Chakrabarti et al., 1993), which may be attributed to the increased hydrophobicity of the resulting molecule. However, it was established that peptides of identical amino acid composition exhibit different activation energies depending upon the distribution of charges within the molecule (Chakrabarti et al., 1993). This indicates that knowledge of amino acid composition alone is insufficient to predict activation energies for insertion into membranes (Chakrabarti et al., 1992; Chakrabarti et al., 1993).

6. What is the biological relevance of the mechanisms for amino acid and modified amino acid permeation?

A. Protein translocation

The mechanisms of amino acid and modified amino acid translocation are also pertinent to the means by which peptides insert into and translocate across lipid

bilayers *in vivo*. Simple diffusion of amino acids is clearly too slow to permit sufficient protein translocation rates to allow for cell growth (Chakrabarti and Deamer, 1992). It follows that a different mechanism must be in place so that potentially charged amino acid side chains are able to overcome the Born energy barrier. One possibility is that ΔpH are involved, so that modified amino acids and peptides would permeate across the membrane in response to ΔpH. The same would also hold true for peptide signal sequences, which are lipophilic weak bases (Gierasch, 1989). These would then be able to permeate as the neutral form, with a net accumulation on the acidic side of the membrane. Some evidence to support this view is that ΔpH may exist in the endoplasmic reticulum (Rees-Jones and Al-Awqati, 1984; Thevenod et al., 1989) and that ΔpH do exist in chloroplasts and may be involved in thylakoid protein translocation (Cline et al., 1992). Clearly, net translocation of signal sequences and some peptides as neutral species appears possible in a variety of natural situations.

B. Origin of life

A final point of interest concerns the relevance of membrane permeability to the origin of membrane transport processes in cells on the early Earth. It is likely that the chemical components and conditions necessary to allow for the formation of lipid vesicles or "protocells" existed on the prebiotic Earth and these presumably served as the progenitors for the first living cells (Deamer, 1986; Morowitz et al., 1988; Morowitz et al., 1991). A key question in this field concerns establishing mechanisms by which such primitive cells could have accumulated essential nutrients without membrane proteins to assist in translocation. There are several mechanisms by which a ΔpH can be produced in simulated protocells under prebiotic conditions, and many potential nutrients are in fact weak acids or bases (Deamer, 1992). It follows that permeation of the neutral species, in the absence of any "accessory" proteins and utilizing just proton gradients as the driving force, provides a mechanism by which the first cells may have accumulated precursors (possibly including modified amino acids) required for growth. Thus, permeation of membranes by the neutral form of amino acids or peptides in response to ΔpH may represent an ancient transport system for nutrient accumulation employed by cells long before the evolution of transport proteins.

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