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Permeation of Membranes by the Neutral Form of Amino Acids and Peptides: Relevance to the Origin of Peptide Translocation

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Abstract. The flux of amino acids and other nutrient solutes such as phosphate across lipid bilayers (liposomes) is 10^5 slower than facilitated inward transport across biological membranes. This suggests that primitive cells lacking highly evolved transport systems would have difficulty transporting sufficient nutrients for cell growth to occur. There are two possible ways by which early life may have overcome this difficulty: (1) The membranes of the earliest cellular life-forms may have been intrinsically more permeable to solutes; or (2) some transport mechanism may have been available to facilitate transbilayer movement of solutes essential for cell survival and growth prior to the evolution of membrane transport proteins. Translocation of neutral species represents one such mechanism. The neutral forms of amino acids modified by methylation (creating protonated weak bases) permeate membranes up to 10^{10} times faster than charged forms. This increased permeability when coupled to a transmembrane pH gradient can result in significantly increased rates of net unidirectional transport. Such pH gradients can be generated in vesicles used to model protocells that preceded and were presumably ancestral to early forms of life. This transport mechanism may still play a role in some protein translocation processes (e.g., for certain signal sequences, toxins and thylakoid proteins) in vivo.

Key words: Peptides — Amino acid — Weak bases — Transmembrane pH gradient — Liposome — Biogenesis — Permeability

Introduction

The permeability of membranes to amino acids (and other nutrients) is of interest because of their importance in cell function. Currently, cells employ specialized membrane transport proteins to provide transport of solutes critical for cell function. How did primitive cells accumulate nutrients prior to the evolution of these transport proteins? It can be calculated that the amino-acid flux rates across the membranes of simple microorganisms such as *E. coli* must be approximately 10^{-2} nmol s^{-1} cm^{-2} in order for these bacteria to double their protein content in 20 min (their doubling time, assuming that an average amino acid has a molecular weight of 150). The flux rates for amino acid and phosphate permeation across liposomal membranes are approximately 10^{-7} nmol s^{-1} cm^{-2} at concentrations in the range of 10–100 mM (Chakrabarti and Deamer 1992). Thus, the first cells would have taken on the order of 4 years to double their protein content if they relied exclusively upon unfacilitated permeation of zwitterionic amino acids for the accumulation of precursors required for growth, even at nutrient concentrations much higher than were likely to have been available. This is clearly too slow to allow for maintenance of cellular structure, let alone growth. Some mechanism must therefore have been available to facilitate the transbilayer trans-

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Abbreviations: LUV, large unilamellar vesicle; ΔpH, transmembrane pH gradient; PAH, polyaromatic hydrocarbon

port of nutrients prior to the evolution of membrane transport proteins. An obvious possibility is that primitive membranes were intrinsically more permeable to polar and ionic solutes than are the highly evolved membranes of contemporary organisms. For instance, primitive lipid-like amphiphilic molecules may have been composed of shorter hydrocarbon chains, or the particular mixture of self-assembled amphiphiles may have been more permeable. An alternative, which we will explore here, is that translocation of nutrients in the form of the neutral species of weak acids and bases provides a mechanism by which ionic solutes could permeate bilayers even if their intrinsic permeability was in the range of contemporary membrane lipids.

Permeability of Membranes to Charged and Neutral Molecules

Amino acids can be chemically modified by creating amides or methyl ester derivatives of their carboxyl groups. The modified compounds are then weak bases which become neutral species at basic pH values. Lipid bilayers are relatively impermeable to zwitterionic amino acids, with measured permeability coefficients (P) in the range of 10^{-12} cm s^{-1} (Chakrabarti and Deamer 1992). The neutral form of modified amino acids permeates lipid bilayers 10^{10} times faster ($P = 10^{-2}$ cm s^{-1}) than unmodified (zwitterionic) amino acids (Chakrabarti et al. 1992).

A significant characteristic of permeant weak bases is that they respond to transmembrane pH gradients (ΔpH) by undergoing net translocation across membranes, followed by accumulation in proportion to the magnitude of the pH gradient. Translocation of weak bases in response to ΔpH has been previously demonstrated for amine uptake in chloroplasts (Crofts 1967), and in liposome systems for fluorescent amines (Deamer et al. 1972), biogenic amines (Schuldiner et al. 1978), acidic phospholipids (Hope et al. 1989), and various drugs (Madden et al. 1990). This phenomenon can result in large transbilayer concentration gradients, because a pH gradient across a liposome membrane (acidic interior) traps the neutral membrane-permeable species in the charged (protonated) form after they traverse the bilayer. Hence, the weak base accumulates in the acidic compartment enclosed by the membrane. It is straightforward to show that, in the absence of membrane partitioning effects, lipophilic amines will be accumulated across membranes with an acidic interior to achieve inside/outside concentration ratios which correspond to the inside/outside concentration ratios of protons. (See Fig. 1.) Thus, a gradient of 1 pH unit results in internal concentrations of weak bases which are approximately 10 times higher than external values (Deamer et al. 1972; Madden et al. 1990; Chakrabarti et al. 1992).

Unmodified zwitterionic amino acids could also translocate via the neutral form. However, only a very small proportion of the amino acids is present in the neutral form at any given instant, depending on the pH (Fig. 2; Chakrabarti 1994). The small size of this neutral population (theoretically 10^6–10^8 less than the charged forms) could account for the much slower permeation rates observed for zwitterionic amino acids when compared to modified amino acids, which are predominantly in the neutral form at basic pH values (Chakrabarti and Deamer 1992; Chakrabarti et al. 1992; Chakrabarti 1994).

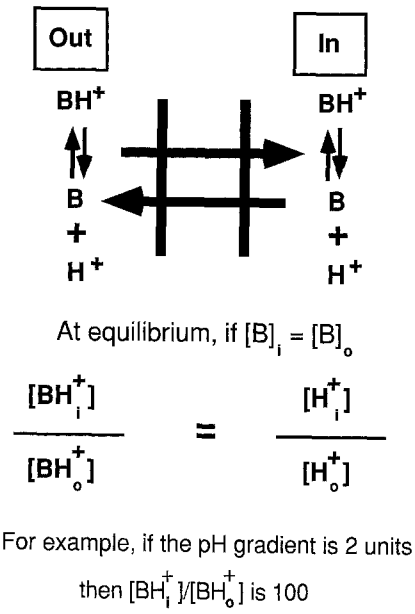

Fig. 1. Redistribution of a weak base in response to a transmembrane pH gradient, where *B* represents the neutral form of the weak base of interest and the subscripts $_i$ and $_o$ refer to the inside and outside of the membrane.

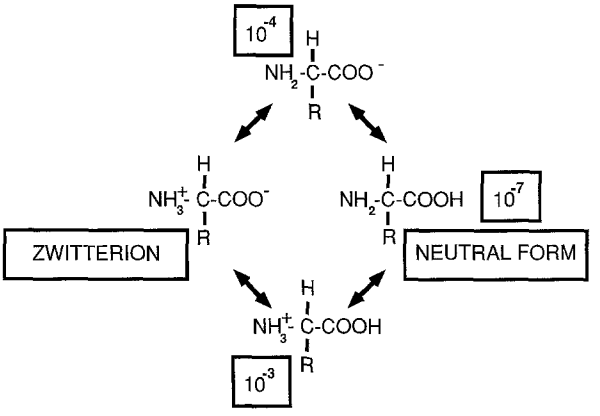

Fig. 2. Equilibrium between the zwitterionic, charged, and neutral forms of a typical amino acid, where *R* represents the side chain. Assuming that $pKa_1 = 3$, $pKa_2 = 10$, and that the pH = 7, the proportion of the population in each form can be estimated using the Henderson-Hasselbalch equation. 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 and Chakrabarti 1994).

Biological Systems Where Neutral Form Transport Can Occur

While many of the steps involved in the translocation of nascent peptides are now known, the energy source for their movement across biological membranes is still unclear (Gierasch 1989; Rapoport 1992). In fact, the driving force for this process was recently described as being "still completely mysterious" (Rapoport 1992). Recent work suggests that permeation of lipid bilayers by the neutral form of peptides may account for some of this transport (Chakrabarti et al. 1992, 1994). Signal sequences are typically lipophilic weak bases, and therefore fulfill the criteria required for pH-dependent translocation (Gierasch 1989). Given that ΔpH may exist in the endoplasmic reticulum (Rees-Jones and Al-Awqati 1984; Thevenod et al. 1989), net translocation of signal sequences and some peptides via permeation of the neutral species appears possible.

Recent research results indicate that this mechanism of transbilayer movement may serve as a "backup" or redundant driving force for the insertion/translocation of some proteins and signal sequences, but only if a ΔpH exists and the proteins normally associated with translocation are absent. It has been observed by Cline et al. (1992) that the transport of certain thylakoid lumen-resident proteins (OE23 and OE17; the 23- and 17-kDa subunits of the oxygen-evolving complex) is absolutely dependent on the presence of a ΔpH, with no ATP requirement at all. However, the mechanism by which a ΔpH could facilitate the translocation of such polypeptides is not clear (Cline et al. 1992). Given the nature of the thylakoid ΔpH (inside acidic), translocation of some of the thylakoid proteins in response to a ΔpH appears a distinct possibility.

New research by Maduke and Roise (1993) studying the import of mitochondrial presequences into liposomes has shown that such presequences can translocate across pure phospholipid bilayers. The proteins normally associated with translocation and the creation of a hydrophilic translocation core were not required (Rapoport 1992). The presence of a membrane potential across the bilayer was found to be the driving force for this translocation (Maduke and Roise 1993). The effects of ΔpH on this system were not determined. However, it appears quite possible that this presequence may translocate in response to ΔpH as well. (See Chakrabarti et al. 1992 for a comparison of peptide translocation in response to a membrane potential and a ΔpH.) This lends further support to the idea that certain peptides can translocate across bilayers as an inherent property of their structures simply on the basis of interactions with the pH/charge concentration gradients that exist across many cell membranes.

The transport phenomena described here may play a role in the translocation of peptides in other biological phenomena as well. Charged amino acids, such as lysine, have been implicated in the localization of secreted and membrane proteins (Boyd and Beckwith 1990). This localization was attributed to interactions of the basic amino acids with the acidic head groups of phospholipids or to interactions with the electrochemical gradient across membranes (Boyd and Beckwith 1990; Skerjanc 1990). Recent work (Chakrabarti et al. 1994) indicated that ΔpH could drive the transbilayer movement and anchoring of peptides or possibly signal sequences containing lysine residues. This work also found that the charge distribution within molecules of identical chemical composition can have dramatic effects upon the rates of translocation observed (Chakrabarti et al. 1994).

It is noteworthy that other peptides, including several toxins, require a ΔpH in order to be translocated across membranes in vivo (Parker et al. 1990). For example, the enzymatically active part of diphtheria toxin only translocates in the presence of an inwardly directed ΔpH (Olsnes et al. 1988). In addition, several natural peptides responsible for a variety of cellular functions have been observed to accumulate into large unilamellar egg phosphatidylcholine liposomes with a ΔpH (inside acidic; Chakrabarti and Cullis, unpublished observations). These peptides were all hydrophobic weak bases with no acidic functions. The ability of these and other peptides to cross bilayers in vivo in response to ΔpH may be related to mechanisms of action and modes of storage and release.

Protocells, Generation of Prebiotic pH Gradients, and Early Membrane Transport

It is likely that the chemical components necessary to form lipid vesicles or "protocells" existed on the prebiotic Earth (Deamer and Oro 1980; Deamer 1992b). Such protocells would have arisen spontaneously from primitive lipidlike amphiphiles that were either of terrestrial or meteoritic origin (Deamer 1986; Deamer and Pashley 1989; Morowitz et al. 1991) and served as the progenitors for the first living cells (Morowitz et al., 1988, 1991; Morowitz 1992). The creation of protocells confers several significant advantages including (1) the potential for the generation of transbilayer concentration gradients, particularly electrochemical gradients; (2) an amphiphilic environment where both polar and nonpolar solutes can exist in the aqueous and lipid phases, respectively; and (3) a stable size range. Vesicles larger than some optimum size will break down, while smaller vesicles will fuse to form larger vesicles (Deamer and Oro 1980; Morowitz et al. 1991).

It has also been previously established that amino acids and peptides could be synthesized or were present as a consequence of cometary or meteoritic infall using models of the prebiotic conditions on the early Earth (Nooner et al. 1977; Deamer 1992b). The question then

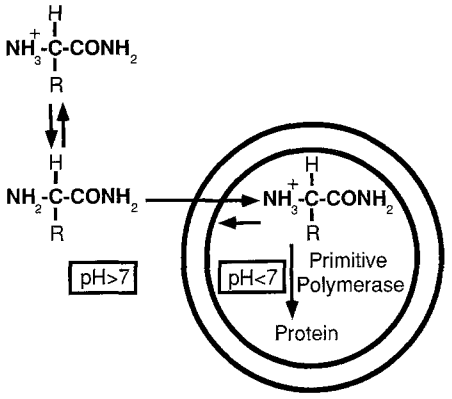

Fig. 3. Uptake of modified amino acids into lipid vesicles with a transmembrane pH gradient (ΔpH, acidic inside). The driving force for the uptake of modified amino acids would be twofold: (1) Redistribution of the weakly basic amino acids in response to the ΔpH and (2) removal of free amino acids from the interior of the vesicle via the polymerization of amino acids to create proteins by a primitive polymerase (modified from Stillwell 1976).

arises as to how the first cells could have accumulated amino acids, small peptides, and other nutrients without membrane proteins to assist in translocation.

Early work by Stillwell (1976) first demonstrated that amino acid flux based on diffusion alone was too slow to allow cellular metabolism to be established. He suggested a facilitated diffusion model where amino acids formed imines with water-soluble aldehydes present in the prebiotic oceans. The imines spread the amino acid charge over a larger area, creating more lipophilic molecules which could diffuse more rapidly through membranes. This chemical coupling would therefore have allowed for faster rates of amino acid accumulation by the first cells.

Another possibility presented in this work is that prebiotic ΔpH may have served as the driving force for amino acid/peptide translocation and concentration. There are several mechanisms by which ΔpH can be produced in simulated protocells under prebiotic conditions. For example, encapsulation of ferrocyanide within liposomes results in the generation of ΔpH (basic inside) upon illumination (Deamer and Harang 1990). Also, polyaromatic hydrocarbon (PAH) derivatives can generate proton gradients (acidic inside) in vesicles under certain conditions (Deamer 1992a). Both ferrocyanide and PAH derivatives were presumably present on the prebiotic Earth and could have been incorporated in primitive protocells. Many potential nutrients are weak acids or bases (Deamer and Oro 1980). It follows that permeation of the neutral forms of these nutrients, 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 and concentrated precursors required for growth. (See Fig. 3; Deamer and Oro 1980.) This mechanism may have acted synergistically with the imine formation suggested by Stillwell (1976).

It is also intriguing to consider the implications of the generation of ΔpH for the origin of chemiosmotic processes. Chemiosmotic production of ATP is shared by most life-forms existing today, indicating a common origin. If ΔpH were utilized for nutrient accumulation by primitive cells on the Earth, the first cells to develop mechanisms to continuously generate and maintain such gradients would have had a significant selective advantage. The evolution of proteins to take advantage of ΔpH for energy production would have led to the contemporary chemiosmotic mechanism. Transport of neutral species in response to ΔpH would then have been gradually relegated to a backup or supporting role in protein translocation. Thus, permeation of membranes by the neutral form of peptides in response to ΔpH may represent a transport mechanism that has been continuously employed by cells since the origin of life.

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