Channel-Mediated Transport of Glucose across Lipid Bilayers

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Facilitated transport of small hydrophilic molecules across cell membranes constitutes an essential metabolic process in eukaryotic organisms. Hydrophilic substances such as glucose can traverse cell membranes only through the action of specific transport proteins.[1] Although significant progress has recently been made toward the design of artificial transmembrane channel structures which can, in some instances, mimic the ion transport activity of the naturally occurring counterparts,[2] the *de novo* design of membrane-spanning systems which can mediate the transport of biologically relevant molecules across lipid bilayers has remained an elusive task.[3] Here we report the design, synthesis, and characterization of the first artificial transmembrane pore structure which displays efficient glucose transport activity.

The rationale and design concepts used in the present study are similar to those recently reported for the construction of transmembrane ion channels.[2a] Briefly, appropriately designed hydrophobic cyclic peptides, made up of an even number of alternating hydrophobic D- and L-amino acids, have been shown to adopt a flat, ring-shaped conformation and to stack to form a contiguous β-sheetlike hydrogen-bonded structure spanning the lipid bilayer (Figure 1). A unique advantage of this strategy is that the internal diameter of the tubular ensemble can be simply adjusted by varying the ring size of the peptide subunit employed.[4] Molecular modeling indicated that, for the passage of glucose through the cylindrical cavity of the tubular transmembrane structure, an internal van der Waals pore diameter of >9 Å is required. Therefore, for the task at hand, we set out to examine the utility of a 10-residue peptide subunit which upon self-assembly can produce tubular ensembles having a uniform 10 Å internal diameter (Figure 1).

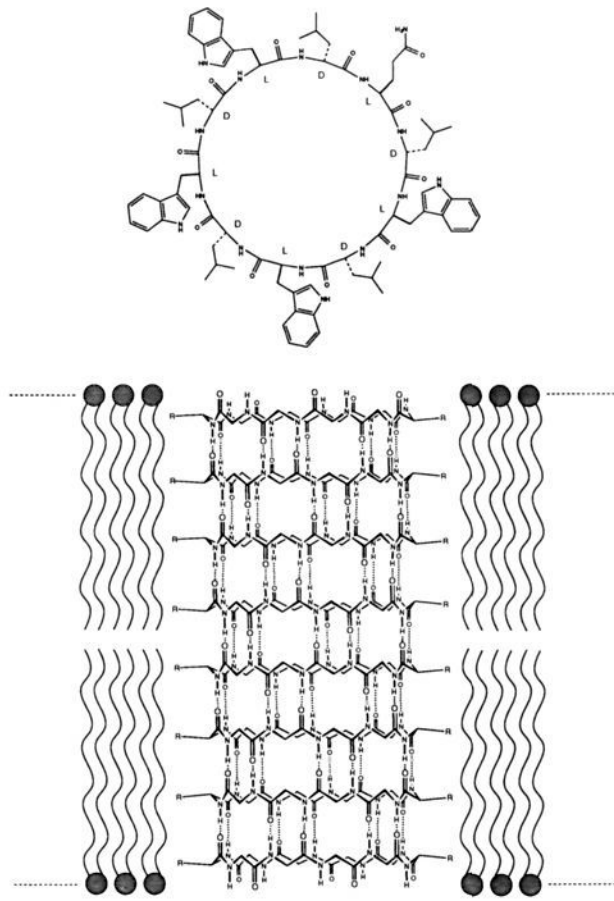

Figure 1. Schematic representation of the self-assembled tubular transmembrane channel structure embedded in a lipid bilayer membrane emphasizing the antiparallel ring stacking, the presence of extensive intersubunit hydrogen-bonding interactions, and side chain−lipid interactions (for clarity, most side chains are omitted). The chemical structure of the peptide subunit is shown on the top (D- or L- refers to the amino acid chirality).

The 10-residue peptide subunit employed in the present study, cyclo[Gln-(D-Leu-Trp)$_4$-D-Leu], is made up largely of tryptophan and leucine residues to favor its partitioning into and self-assembly in lipid bilayers. It was synthesized on solid support[5] and characterized by ^{1}H NMR spectroscopy and mass spectrometry. Addition of the peptide subunit to aqueous suspensions of large unilamellar liposomes effects a rapid incorporation of the peptide into the lipid bilayer. This has been supported by absorption and fluorescence spectrophotometry and gel permeation studies. Formation of a hydrogen-bonded transmembrane channel structure in phosphatidylcholin liposomes has been established by FT-IR spectroscopy.[6] The observed amide-I band at 1625 cm^{-1} and the N−H stretching band at 3272 cm^{-1} are similar to those of the previously characterized peptide nanotubes[2a,4] and strongly support the formation of a tight network of β-sheetlike hydrogen-bonded structures with an average intersubunit distance of 4.8 Å. Formation of transmembrane channels was also inferred from its remarkably high ion transport efficiencies (>10^7 ions·s^{-1}), as indicated by single ion channel recordings using micropatch clamp techniques.[7]

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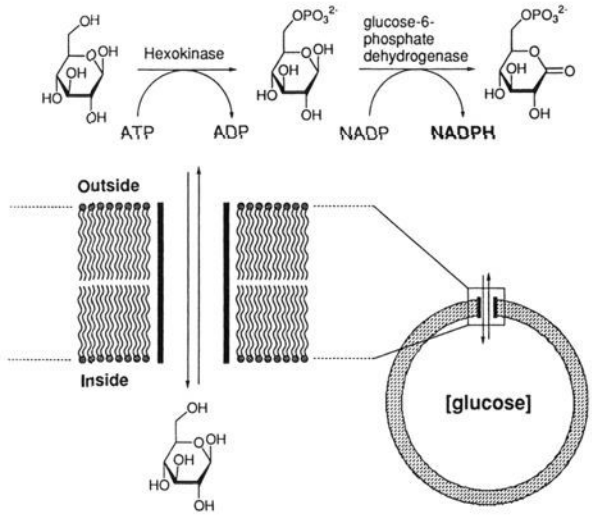

Figure 2. Schematic illustration of channel-mediated glucose transport and the enzyme-coupled assay used to monitor the transport activity. Formation of the transmembrane pore structure(s) initiates glucose efflux from the liposome, which can be directly monitored by measuring the rate of NADPH production. The enzymes and cofactors employed are hydrophilic and thus cannot pass through the lipid membrane and are too large to penetrate the channel structure. Therefore, only the released glucose can undergo the enzymatic reaction.

Glucose transport activity was studied in isotonic solutions using glucose-entrapped unilamellar lipid vesicles.[8,9] The transport phenomenon was monitored spectrophotometrically at 340 nm for the production of NADPH using an enzyme-coupled assay[10,11] (Figure 2). The transport of glucose initiated by the addition of various amounts of the channel-forming peptide to the glucose-entrapped liposomes follows a first-order rate profile with apparent rate constants of 1.2 ± 0.09, 0.74 ± 0.1, 0.48 ± 0.05, and 0.18 ± 0.02 mol(glucose)/mol(peptide)/min for liposomes having initial glucose concentrations of 200, 150, 100, and 50 mM, respectively[11,12] (Figure 3). Unlike in carrier-mediated transport, which must display Michaelis–Menten saturation kinetics, the observed linear relation between

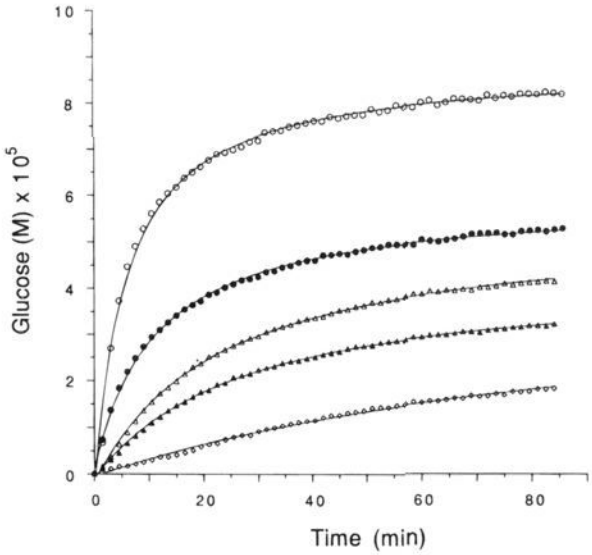

Figure 3. Ensuing glucose efflux on the addition of various amounts of the channel-forming peptide, (○) 15.0×10^{-6}, (●) 11.0×10^{-6}, (△) 7.5×10^{-6}, (▲) 5.6×10^{-6}, and (◇) 3.8×10^{-6} M, expressed in terms of the amount of glucose released as a function of time (sampled at 90 s intervals). The large unilamellar vesicles used in this study contained 200 mM D-glucose. All curves are background corrected to remove any contribution from the nonspecific glucose leakage from the liposomes.[8,11]

transport rate and glucose concentration strongly supports a simple transmembrane channel-mediated diffusion process.[13] Control studies, monitoring the release of entrapped 5(6)-carboxyfluorescein under similar conditions, established that the transport of glucose was due neither to the rupturing of the liposomes nor to the small amounts of DMF (<2%) employed in these studies.[14] Furthermore, neither gramicidin A, a well-known naturally occurring ion channel-forming peptide with an internal diameter of approximately 4.5 Å, nor the very similar ion channel-forming peptide cyclo[Gln-(D-Leu-Trp)$_3$-D-Leu], previously characterized and shown to form channels with approximately 7.5 Å internal diameter,[2a] displays any glucose transport activity under similar assay conditions. Together, the above studies lend strong support for the size-selective, pore-mediated transport of glucose.

In short, the present study provides the first example of an artificial transmembrane channel construct which is capable of an efficient transport of a neutral hydrophilic substrate across lipid bilayers. The present study, in conjunction with the recent high-resolution X-ray structural analysis of a related system,[15] unequivocally establishes the presence of an aqueous, hydrophilic transmembrane pore structure which can be exploited to effect size- and shape-selective molecular transport across lipid membranes. Such systems may have potential utility as novel drug delivery vehicles.

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(8) Unilamellar vesicles, ~150 nm in diameter, were prepared by the reverse-phase evaporation method[9] using 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine (POPS), and cholesterol in the ratio 1:1:0.1:1 in a solution containing 50, 100, 150, or 200 mM D-glucose, 100 mM NaCl, and 50 mM Tris buffer, pH 7.5. Liposomes were gel-filtered using Sephadex G-25 in an isotonic buffer containing 50, 100, 150, or 200 mM sucrose, 100 mM NaCl, and 50 mM Tris buffer, pH 7.5. The liposome preparation was stored at 4 °C and used within 24 h of the synthesis.

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(11) All experiments were performed on a Spectronic-3000 photodiode array spectrophotometer using 3 mL quartz cuvettes placed in the thermojacketed multiple cell holder and held at 27 °C. In a typical experiment the following solutions were sequentially placed in the cuvettes: 750 μL of buffer (300 mM NaCl, 50 mM Tris pH 7.5, 3.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$), 500 μL of the enzyme solution (8 units·mL^{-1} of hexokinase, 16 units·mL^{-1} of glucose-6-phosphate dehydrogenase, 2.5 mM ATP, and 1.3 mM NADP all dissolved in 200 mM NaCl, 50 mM Tris pH 7.5, 3.5 mM $MgCl_2$, and 0.15 mM $CaCl_2$), and 75 μL of the stock liposome solution[8] (2.6×10^{-3} M in phospholipids). Total glucose content in each cuvette was determined by Triton X-100 treatment. Transport was initiated by the addition of an appropriate amount (5, 7.5, 10, 15, or 20 μL) of the peptide solution (1 mM in DMF) to the reference and the sample cuvettes (hexokinase was omitted from the reference sample). For measuring the background (nonspecific glucose leakage from the liposomes), the sample was prepared in an identical fashion, except appropriate amounts of DMF were added in place of the channel-forming peptide. The production of NADPH was monitored at 340 nm for 1.5 h at 90 s time intervals. Because of the high catalytic efficiencies of the enzymes employed, the rate of NADPH production is directly proportional to the rate at which glucose is released from the liposomes.[10]

(12) The apparent rate of glucose transport is, in all likelihood, a gross underestimation of the actual rate of channel-mediated transport because only a minute fraction of the total number of peptides incorporated in the lipid bilayer are assembled, at a given time, in the form of active transmembrane channel structures.

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