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Association of synthetic model peptides with phospholipid vesicles induced by a membrane potential

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Hydrophobic model peptides, consisting of 5 or 6 amino acids and carrying a nett positive charge at the amino terminus, exhibit a dramatically increased association with large unilamellar egg-PC vesicles upon application of a valinomycin-induced K^+ diffusion potential, negative inside. The association of the peptides is largely reversible, apparent from a release of peptide upon dissipation of the membrane potential.

Membrane potential plays an important role in a number of processes involving membrane insertion and transport of proteins. Import of most mitochondrial precursor proteins requires a membrane potential across the inner mitochondrial membrane [1], whereas in protein export in Gram-negative bacteria the membrane potential in several cases facilitates transport [2–4]. Furthermore membrane potential has been implicated in the entry of diphtheria toxin into cells [5] and the formation of colicin channels in membranes [6].

The molecular mechanism by which the membrane potential acts in these processes is largely unknown. One possibility is that it influences the interaction of the polypeptide with the membrane lipids. This hypothesis was supported by black lipid membrane studies which showed that applying a potential of the appropriate polarity can accomplish reorientation of inserted proteins within the bilayer [7]. However, studies on the influence of membrane potential on the interaction of mitochondrial presequences with phospholipid vesicles yielded contradictory results [8,9].

Here we report the first results of a model system approach using synthetic model peptides in combination with phospholipid vesicles, to assess the influence of the membrane potential on lipid–peptide interactions.

Two peptides have been synthesized by solid phase peptide synthesis: a hexapeptide, H_3N^+-Ala-Ile-Met-Leu-Trp-Ala-COO^-, and a pentamerous homologue lacking the isoleucine residue. Criteria for the design of these model peptides were a general hydrophobic character and the containment of a tryptophan residue to enable sensitive detection. After cleavage from the resin by HF, peptides were purified by reversed-phase HPLC on a Si 100 (10 μ) Polyol RP 18 (Serva) column, eluted with linear water-acetonitril gradients containing 0.1% (v/v) trifluoroacetic acid. Methylation of the carboxyl terminus to obtain positively charged peptides was accomplished by the BF_3/MeOH method [10], which was followed by an additional HPLC step, yielding over 97% pure peptides. Peptide concentrations of stock solutions in either DMSO (hexapeptide, $\approx$ 13 mM) or buffer (pentapeptide, $\approx$ 2 mM) were quantified spectrophotometrically at 280 nm using $\epsilon = 5.6\ M^{-1} \cdot cm^{-1}$.

Egg PC was purified from hen egg yolks according to standard procedures. Large unilamellar egg-PC vesicles were prepared by the extrusion technique [11] using 400 nm pore size polycarbonate filters. A transmembrane K^+/Na^+ gradient was generated by preparing the vesicles in 150 mM K_2SO_4, 20 mM Hepes (pH 7.0), after which the external K^+-buffer was replaced by Na^+-buffer by passing the vesicles through a Sephadex G-50 column (1 × 20 cm) eluted with 150 mM Na_2SO_4, 20 mM Hepes (pH 7.0). Sulfate was chosen as anion to reduce permeation of the valinomycin-K^+ complex [12] enabling the generation of stable membrane potentials.

Abbreviations: HPLC, high-performance liquid chromatography; PC, phosphatidylcholine; DMSO, dimethylsulfoxide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; diS-C_2-(5), 3,3'-diethylthiadicarbocyanine iodide; LUV, large unilamellar vesicle.

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Incubation experiments were carried out at room temperature in Na^+-buffer with 1 mM PC and 0.1 mM peptide (at this concentration both peptides are water soluble), either with or without the addition of valinomycin (Boehringer, 1 mg/ml in ethanol) to a 1 : 1000 molar ratio with respect to PC. At various times after starting the incubations 100 μl aliquots were taken and within 15 s centrifuged through 1 ml Sephadex G-50 minicolumns in order to separate vesicle-associated from free peptide [11,13]. The eluates containing the vesicles were subsequently analyzed for phospholipid phosphorus [14] and peptide. The latter was quantified by tryptophan fluorescence measurements (λ_{ex} = 280 nm, λ_{em} = 354 nm) on a Perkin-Elmer LS 5 Luminescence Spectrometer, in 0.5% (w/v) sodium cholate-containing buffer, after it had been established that under these conditions the emission intensity was linearly proportional to the amount of peptide. The binding data determined according to this method contain a maximal inaccuracy of 2 nmol peptide per μmol PC.

Fig. 1 compares the vesicle association of the methylated penta- and hexapeptide in the absence and presence of a membrane potential. Without valinomycin present a very limited level of association is observed under these conditions, irrespective of the vesicles experiencing a K^+/Na^+ ion gradient. Application of a membrane potential, negative inside, by adding valinomycin gives rise to a tremendous increase in the amount of vesicle-associated peptide. This increase is more pronounced for the hexapeptide where it reaches 30% of the total amount of peptide available, than for the less hydrophobic pentapeptide. Control experiments showed that in the absence of an ion gradient valinomycin does not affect peptide-vesicle association. A nett positive charge on the peptide is required for the effect to occur, since the very low level (less than 4 nmol/μmol PC after 2 h) of association of the homologous zwitterionic peptides with the vesicles is not influenced by the presence of a membrane potential.

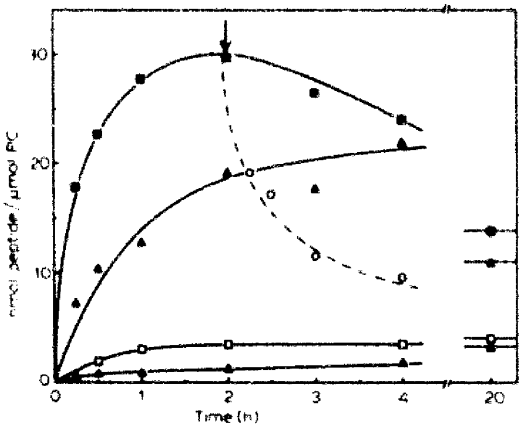

Fig. 1. Time dependence of the association of the methylated pentapeptide (▲, △) and the methylated hexapeptide (■, □) with egg-PC LUVs experiencing a K^+/Na^+ ion gradient in the absence (open symbols) and presence (closed symbols) of valinomycin. At the arrow, tryptophan-N-formylated gramicidin was added to the incubation containing the hexapeptide in order to dissipate membrane potential. The subsequent release of vesicle-associated peptide (○) is represented by the dashed line. Experimental conditions are described in the text.

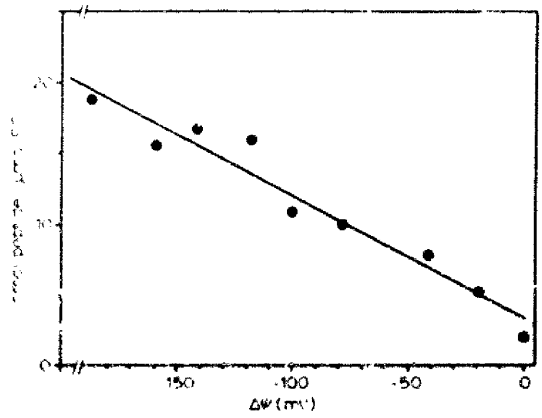

Fig. 2. Dependence of the amount of methylated pentapeptide associated with egg-PC LUVs after 4 h of incubation on the initial value of the applied membrane potential. Incubations and quantification of the associated peptide were carried out as outlined in the text. The calculated best linear fit of the data is depicted assuming a linear correlation between the amount of associated peptide and the initial value of the membrane potential.

The association process is at least partly reversible: dissipation of the membrane potential by the addition of tryptophan-N-formylated gramicidin (0.5 mM in DMSO) to a 1 : 400 molar ratio with respect to PC, causes the release of vesicle-associated peptides with a halftime of 30 min in the case of the hexapeptide (Fig. 1). The tryptophan-N-formylated derivative of gramicidin (prepared according to [15]) instead of the parent molecule was used to avoid contribution to the peptide's tryptophan fluorescence level. Employing diS-C_2-(5) as membrane potential sensitive fluorescent dye [16] tryptophan-N-formylated gramicidin was shown to dissipate the membrane potential completely within 20 min after its addition under the conditions used (data not shown).

The reversibility of the association is also apparent from the time course of the amount of vesicle-associated peptide. The amount of vesicle-associated hexapeptide starts to decline after 2 h of incubation, whereas after 20 h a considerable decrease has occurred for both peptides (Fig. 1). This was parallelled by a slow, peptide-dependent dissipation of the membrane potential (measured by diS-C_2-(5)), which occurred earlier in the case of incubation with the hexapeptide than with the pentapeptide (data not shown).

That indeed the amount of peptide associated with the vesicles depends on the value of the applied membrane potential is substantiated in Fig. 2. Incubation of vesicles with methylated pentapeptide and valinomycin was carried out at various external K^+ concentrations

(while keeping the sum of external K^+ and Na^+ constant), thus generating a series of different initial values of membrane potential. The amount of associated peptide was found to be proportional to the calculated Nernst potential ($\Delta\psi_{initial} = (RT/zF)\ln([K^+]_{out}/[K^+]_{in})$).

In conclusion a valinomycin-induced membrane potential, negative inside, applied to egg-PC LUVs, causes vesicle association of positively charged peptides, which exhibit scarcely any affinity for these vesicles in its absence. The extent of association depends both on the hydrophobicity of the peptide and the initial value of the applied potential. In addition the association was shown to be largely reversible. Presently the question of the precise localization of the vesicle-associated peptides is addressed.

The data clearly demonstrate that membrane potential is capable of greatly affecting lipid-peptide interactions and as such bear relevance for, e.g., mitochondrial protein import. Another area for which these results may be of interest is the binding of regulatory peptides to their target membranes. [17]. Furthermore the selective and reversible adsorption of cationic peptides to vesicles upon introduction of a membrane potential could possibly generate applications in peptide isolation and purification.

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