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Association of an acylated model peptide with DPPC–DPPS lipid membranes☆

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Abstract

The interaction between a small positively charged peptide with a N-terminally linked acyl chain and dipalmitoylphosphatidylcholine–dipalmitoylphosphatidylserine (DPPC–DPPS) lipid membranes has been studied by means of fluorescence resonance energy transfer. Two different lipid compositions were used: a neutral membrane (100 mol% DPPC), and a negatively charged membrane (30 mol% DPPS in DPPC). The fluorescence resonance energy transfer results reveal that the peptide associates with both types of membranes. Furthermore, it is found that the slope of the titration curve for the negatively charged membranes is much steeper than that for the neutral membranes. This indicates a higher binding affinity of the acylated peptide towards negatively charged lipid membranes as compared with neutral lipid membranes.

Keywords: Acylated peptide; Peptide–membrane association; Negatively charged membranes; Fluorescence resonance energy transfer

Peptides and proteins control a large number of physiological processes in the body. The therapeutic potential of these macromolecular agents is therefore enormous (Krogsgaard-Larsen and Bundgaard, 1991). One of the largest drawbacks of peptide/protein delivery is the very fast degradation of these compounds in the blood stream. The potential use of biologically active peptides therefore requires new ways to overcome the fast degradation of the labile peptides in the human body. One way to solve the degradation problem is to chemically modify and introduce new structures in the peptides, i.e. peptide prodrugs and analogs. This can be achieved by coupling a stabilising peptide sequence to a pharmacologically active peptide, and in that way make the peptide less accessible for enzymatic degradation (Larsen, 1999). Another way to chemically modify pep-

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tides/proteins is to acylate the peptides/proteins. This conjugation is usually carried out by forming an amide bond between the fatty acid and the amino group in the peptide/protein (Debono et al., 1995; Pool and Thompson, 1998; Veuillez et al., 1999). Interestingly, several naturally occurring membrane associated proteins and peptides are acylated (Silvius, 1999). It is assumed that fatty-acid acylation is involved in the anchoring of proteins and peptides to membranes (Peitzsch and McLaughlin, 1993; McLaughlin and Aderem, 1995). The acylation renders the peptide/protein more lipophilic and thus increases its ability to penetrate into and associate with membranes (Muranishi et al., 1991). It has been reported that acylation of insulin can improve insulin stability in the small intestinal fluid as well as in the intestinal mucosa, and in addition can lead to an enhanced gastrointestinal absorption (Asada et al., 1994).). Furthermore, acylated insulin has been shown to bind to albumin and in this way protract the effect of insulin (Kurtzhals et al., 1995). This concept could be generally applicable to control and prolong the therapeutic profile of peptide/protein drugs. Recently, it has been found that the antidiuretic potency of a reversible lipidized dipalmitoyl desmopressin is increased by a factor of 250 as compared with the natural unmodified desmopressin (Wang et al., 1999). The enhanced potency of the lipidized desmopressin is ascribed to a slow elimination and prolonged tissue retention.

In this study, we investigate the interaction between a small acylated model peptide and liposomes composed of zwitterionic phosphatidylcholine lipids and negatively charged phosphatidylserine lipids. The aim of this study is to gain deeper insight into the membrane association of acylated peptides in terms of changes in the membrane structure, on the one hand, and in the secondary structure of the peptide, on the other. We investigate how the membrane association is affected by the lipid composition of the liposomal membrane. In particular, such results are of importance for the potential use of liposomes as drug delivery systems for membrane-associated peptides.

Multilamellar liposomes composed of dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylserine (DPPS), and a fluorescent headgroup-labelled lipid, *N*-(5-dimethylaminonaphtalene - 1 - sulfonyl) - 1,2 - dihexadecanoyl - *sn*-glycero-3-phosphoethanolamine (dansyl-DHPE) were made by dispersion of a dried lipid film in a buffer solution containing 10 mM MES, 50 mM KCl and 1 mM NaN_3, (pH 5.8). Large unilamellar liposomes were made by standard extrusion techniques (Hope et al., 1985). Myristoyl-HWAH-PGGHHA-amide (acylated peptide) of high purity was synthesised by Commenwealth Biotechnologies, (Richmond, VA.).

Fluorescence resonance energy transfer measurements were performed using a SLM DMX-1100 fluorometer (SLM Instruments, Urbana, IL). To monitor fluorescence resonance energy transfer between tryptophan (donor) and dansyl-DHPE (acceptor), the quenching of tryptophan was followed upon titration with liposomes containing 5 mol% dansyl-DHPE. Tryptophan was excited at 280 nm and emission was recorded at 350 nm. The emission of the acceptor was followed at 506 nm. In parallel with the acceptor-labelled liposomes, blank liposomes without fluorescent acceptor probes were used as titrants. The energy transfer efficiency, E, was calculated as:

$$E = 1 - F_{DA}/F_D$$

where F_{DA} is the fluorescence intensity of tryptophan in the presence of acceptor-labelled liposomes, and F_D is the fluorescence intensity of tryptophan in the absence of acceptor (blank liposomes). The fluorescence intensities were corrected for dilution and absorption effects (Lakowicz, 1999; Pedersen et al., unpublished observations).

Fig. 1 shows the tryptophan fluorescence of the acylated peptide during titration with liposomes composed of 95 mol% DPPC and 5 mol% dansyl-DHPE. Titration with liposomes containing dansyl-DHPE (acceptor probes) decreases the fluorescence intensity due to quenching of tryptophan by acceptor. When acylated peptide is titrated with blank liposomes, the fluorescence intensity increases. The enhancement of fluores-

cence upon membrane association is seen for several extrinsic proteins upon binding (Devaux and Seigneuret, 1985). This enhancement reflects a decrease of the polarity of the tryptophan environment upon binding to the liposomes. This effect can be due to a direct interaction of tryptophan with the phospholipid membrane or to a conformational change of the peptide resulting in a lesser accessibility of tryptophan to water (Devaux and Seigneuret, 1985; Lakowicz, 1999).

In Fig. 2, the fluorescence resonance energy transfer efficiency, E, is shown as a function of dansyl-DHPE acceptor concentration. The titration curves reveal that the peptide associates with liposomes composed of negatively charged lipids (30 mol% DPPS in DPPC) as well as with liposomes composed of neutral zwitterionic DPPC lipids. However, the slope of the titration curves for the negatively charged membranes is much steeper than the slope for the neutral membranes, indicating a higher binding affinity towards the lipid membranes containing the negatively charged DPPS lipids. The two curves reach almost the same level for high concentrations of lipid to peptide, suggesting that the membranes will eventually bind the same amount of peptide. The fluorescence resonance energy transfer is strongly dependent on the distance between donor and acceptor. The high efficiency values therefore indicate a close proximity between the donor and the acceptor, suggesting that the association and positioning of the peptide take place close to the headgroup region of the lipid membrane.

In order to obtain a deeper understanding of the positioning of the peptide at the membrane/water interface, we are currently conducting research using fluorescent lipids labelled in different positions relative to the headgroup-labelled lipid used in the present study. Whether the association of the peptide with the lipid membranes is due to a strong tendency of the hydrophobic acyl chain to insert into the membrane or the peptide positions itself at the membrane/water interface is currently being investigated by means of fluorescence spectroscopy, calorimetry and Fourier-transform infrared spectroscopy.

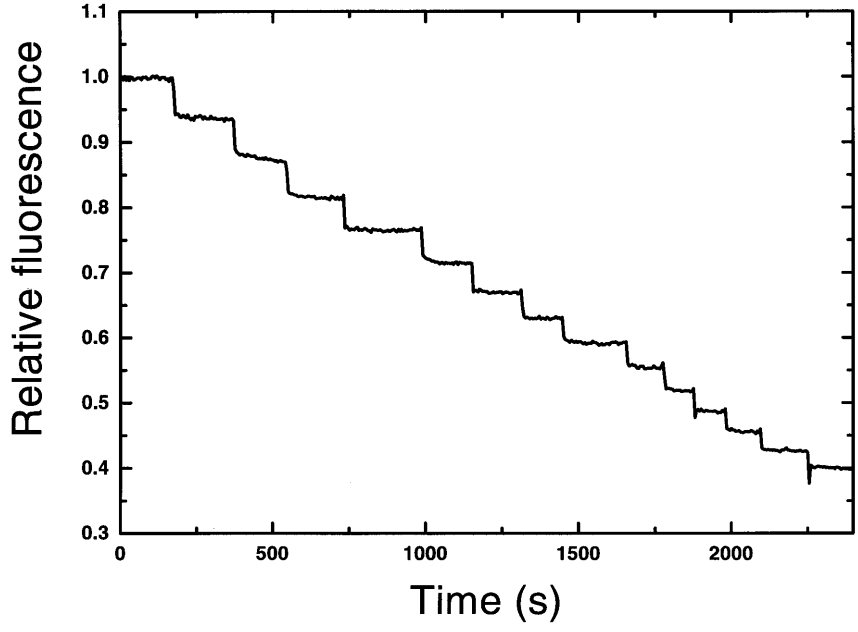

Fig. 1. Fluorescence of acylated peptide upon titration with liposomes containing 95 mol% DPPC and 5 mol% dansyl-DHPE. Each step on the curve corresponds to addition of 100 nmol lipids. The final lipid:peptide ratio is 37:1.

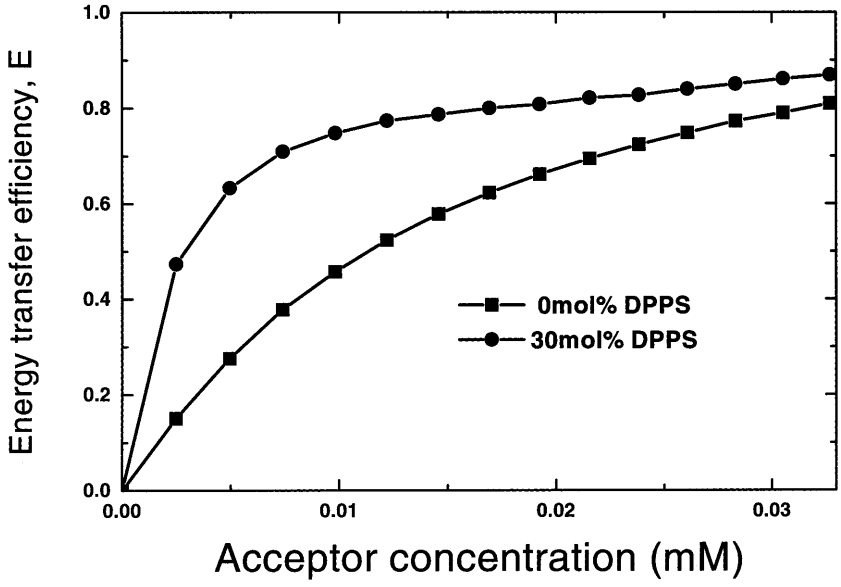

Fig. 2. Fluorescence resonance energy transfer efficiencies for two different lipid membrane compositions (100 mol% DPPC and 30 mol% DPPS in DPPC) as a function of dansyl-DHPE (donor) concentration. The final lipid:peptide ratio is 37:1.

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References

Asada, H., Douen, T., Mizokoshi, Y., Fujita, T., Murakami, M., Yamamoto, A., Muranishi, S., 1994. Stability of acyl derivatives of insulin in the small intestine: relative importance of insulin association characteristics in aqueous solution. Pharm. Res. 11, 1115–1120.

Debono, M., Turner, W.W., LaGrandeur, L., Burkhardt, F.J., Nissen, J.S., Nichlos, K.K., Rodriguez, M.J., Zweifel, M.J., Zeckner, D.J., Gordee, R.S., Tang, J., Parr, T.R., Jr, 1995. Semisynthetic chemical modification of the antifungal lipopeptide echinocandin B (ECB): structure–activity studies of the lipophilic and geometric parameters of polyarylated acyl analogs of ECB. J. Med. Chem. 38, 3271–3281.

Devaux, P.F., Seigneuret, M., 1985. Specificity of lipid–protein interactions as determined by spectroscopic techniques. Biochim. Biophys. Acta 822, 63–125.

Hope, M.J., Bally, M.B., Webb, G., Cullis, P.R., 1985. Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. Biochim. Biophys. Acta 812, 55–65.

Krogsgaard-Larsen, P., Bundgaard, H. (Ed.), 1991. A Textbook of Drug Design and Development. Harwood Academic Publishers GmbH, Chur, Switzerland.

Kurtzhals, P., Havelund, S., Jonassen, I., Kiehr, B., Larsen, U.D., Markussen, J., 1995. Albumin binding of insulin acylated with fatty acids: characterization of the ligand–protein interaction and correlation between binding affinity and timing of the insulin effect *in vivo*. Biochem. J. 312, 725–731.

Lakowicz, J.R., 1999. Principles of Fluorescence Spectroscopy, 2nd ed. Kluwer Academic/Plenum, Publishers, New York.

Larsen, B.D., 1999. Patent WO 99/46283, 16 September.

McLaughlin, S., Aderem, A., 1995. The myristoyl–electrostatic switch: a modulator of reversible protein–membrane interactions. TIBS 20, 272–276.

Muranishi, S., Sakai, A., Yamada, K., Murakami, M., Takada, K., Kiso, Y., 1991. Lipophilic peptides: synthesis of lauroyl thryptropin-releasing hormone and its biological activity. Pharm. Res. 8, 649–652.

Peitzsch, R.M., McLaughlin, S., 1993. Binding of acylated peptides and fatty acids to phospholipid vesicles: pertinence to myristoylated proteins. Biochemistry 32, 10436–10443.

Pool, C.T., Thompson, T.E., 1998. Chain length and temperature dependence of the reversible association of model acylated proteins with lipid bilayers. Biochemistry 37, 10246–10255.

Silvius, J.R., 1999. Lipid modification of intracellular signal-transducing proteins. J. Liposome Res. 9, 1–19.

Veuillez, F., Deshusses, J., Buri, P., 1999. Synthesis and characterization of an acylated di-peptide (Myr-Trp-Leu) with modified transmucosal transport properties. Eur. J. Pharm. Biopharm. 48, 21–26.

Wang, J., Shen, D., Shen, W.C., 1999. Preparation, purification, and characterization of a reversibly lipidized desmopressin with potentiated anti-diuretic activity. Pharm. Res. 16, 1674–1679.