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Interaction of a hydrophobic model peptide and its fatty acid derivative with lipid vesicles

M. Joseph and R. Nagaraj

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

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The interaction of a peptide [Lys-Phe-Phe-Phe-Ile-Ile-Trp-OCH_3] and its fatty acid derivatives [Lys-(ε-palmitoyl)-Phe-Phe-Phe-Ile-Ile-Trp-OCH_3 and Lys-{ε-12-(9-anthroyloxy)stearic acid}-Phe-Phe-Phe-Ile-Ile-OCH_3] with model membranes was investigated by fluorescence spectroscopy. The emission characteristics of the Trp fluorophore indicated that only the peptide with the fatty acid chain is associated with lipid vesicles. Quenching experiments with spin probes suggest an orientation for the fatty acylated peptide wherein the fatty acid chain is perpendicular to the bilayer surface and the peptide chain parallel to the bilayer surface.

Hydrophobic peptide; Fatty acid acylation; Fluorescence spectroscopy; Fluorescence quenching

1. INTRODUCTION

Integral membrane proteins without exception have at least one segment comprising a contiguous stretch of hydrophobic amino acids [1,2]. Studies using recombinant DNA techniques and in vitro reconstitution experiments have indicated that the hydrophobic stretch is absolutely essential for membrane association [3–6]. Thermodynamics favours the spontaneous partitioning of a contiguous stretch of hydrophobic amino acids, particularly in an α-helical conformation, into the lipid bilayer [7,8]. Hence, a stretch of hydrophobic amino acids would be able to 'anchor' to a membrane surface. However, in recent years, fatty acylation of membrane proteins with palmitic acid has been observed as a post-translational modification [9,10]. The fatty acid is located near the hydrophobic stretch of amino acids. It is not clear why this modification is necessary as a hydrophobic stretch of amino acids would be sufficient for membrane anchorage. In order to elucidate the manner in which a covalently linked fatty acid would modulate the association of a hydrophobic peptide with lipid components of membranes, we have studied the interaction of the peptides, Lys-Phe-Phe-Phe-Ile-Ile-Trp-OCH_3 (**1**), Lys-(ε-palmitoyl)-Phe-Phe-Phe-Ile-Ile-Trp-OCH_3 (**2**) and Lys-{ε-12-(9-anthroyloxy)stearoyl}-Phe-Phe-Phe-Ile-Ile-OCH_3 (**3**), with unilamellar lipid vesicles of phosphatidylcholine. The sequence Phe-Phe-Phe-Ile-Ile is part of the membrane-anchoring sequence of the VSV G protein [11].

Correspondence address: R. Nagaraj, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500007, India

2. EXPERIMENTAL

2.1. *Synthesis of peptides*

Peptides **1**–**3** were synthesized by solution-phase methods. Details of synthetic protocols for Boc-Lys-(ε-carbobenzoxy)-Phe-Phe-Phe-Ile-Ile-OCH_3 are described in [12]. The above peptide was saponified to generate the free carboxy terminus acid or treated with formic acid and Pd black [13] to remove the ε-NH_2-protecting group of Lys. The peptide acid was coupled to TrpOCH_3 in DMF in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The *N*-hydroxysuccinimide esters of palmitic and 12-(9-anthroyloxy)stearic acids were added to the peptide with the free ε-NH_2 group. The protected peptides were purified by column chromatography on silica gel with mixtures of chloroform and methanol as eluants. The compositions of the peptides were confirmed by quantitative amino acid analysis and gas-liquid chromatography.

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The pure, protected peptides were treated with 85% formic acid to yield peptides **1–3**.

2.2. *Preparation of lipid vesicles and fluorescence measurements*

Small unilamellar vesicles (SUVs) were prepared by sonication of an aqueous dispersion of purified egg phosphatidylcholine (PC) in Hepes buffer (pH 7.4). Fluorescence measurements were recorded on a Hitachi 650-10S fluorescence spectrophotometer. Aliquots of peptides dissolved in methanol were added to lipid vesicles. For the anthroyl fluorophore λ_{ex} = 360 nm and for Trp λ_{ex} = 280 nm.

3. RESULTS AND DISCUSSION

The emission spectrum of the anthroyl fluorophore in peptide **3** in the presence of SUVs of PC, as a function of time, is shown in fig.1. No change in emission characteristics is observed with respect to time. Also, the emission maximum indicates that the anthroyl group is associated with the hydrophobic core of the lipid bilayer [an identical emission spectrum was obtained for 12-(9-anthroyloxy)stearic acid at an equivalent concentration in the presence of PC vesicles]. The emission spectra of the Trp fluorophore in **1** and **2** in the presence of PC vesicles are depicted in fig.2 as a function of time. A large increase in fluorescence is observed only for the fatty acylated peptide **2**. The maximum value of the fluorescence intensity is reached after about 12 h, indicating slow incorporation of the peptide chain into the lipid bilayer. Thus, the fatty acid chain is clearly necessary for the peptide chain to be associated with the lipid bilayer.

The fatty acylated peptide, Lys-(ϵ-palmitoyl)-Phe-Phe-Phe-Ile-Ile-OCH_3, can conceivably associate with lipid vesicles primarily in four different ways as shown in fig.3. The mode of association shown in fig.3d would lead to aggregation of lipid vesicles, particularly at low lipid:peptide ratios. However, no aggregation of lipid vesicles was observed as judged from the 90° light scattering in the presence of fatty acylated peptide. Changes in λ_{max} of Trp in peptide **2** in the presence of lipid vesicles argue against the peptide chain being oriented on the surface of lipid vesicles as depicted in fig.3c. Hence, the two possible orientations of the peptides in the presence of lipid vesicles are as shown in fig.3a,b.

In order to determine the relative orientation of the fluorophore in peptides **1–3** in the lipid bilayer,

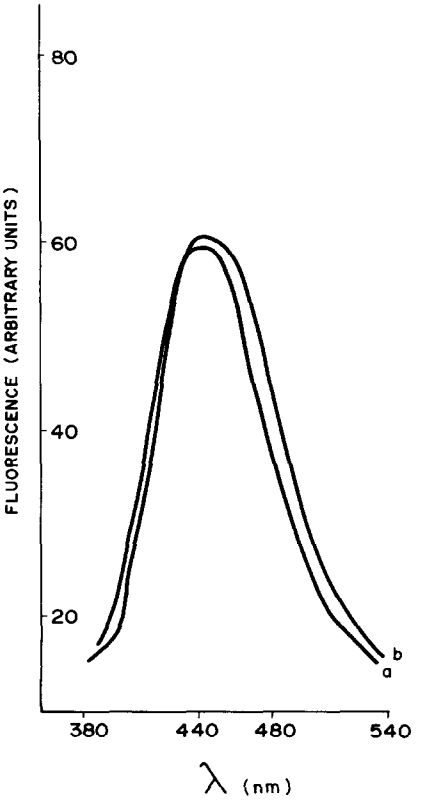

Fig.1. Fluorescence emission spectra of peptide **3** in the presence of lipid vesicles as a function of time. [Peptide] = 6 μM; [lipid] = 250 μM. Spectra: (a) immediately after peptide addition to lipid vesicles; (b) 12 h after peptide addition to lipid vesicles.

quenching of fluorescence by the spin labels 5- and 12-doxylstearic acid was determined. The quenching data are presented in fig.4 in the form of Stern-Volmer plots. The quenching efficiencies of 5- and 16-doxylstearates of the anthroyl fluorophore are similar in the peptide and 12-(9-anthroyloxy)stearic acid, indicating a similar transverse orientation in the lipid bilayer. The Trp fluorophore in peptide **2** is quenched less efficiently than the anthroyl fluorophore by 5- and 16-doxylstearic acids, suggesting that the peptide chain in **2** is not associated with the lipid bilayer in a transverse orientation. The quenching studies thus suggest an orientation for the acylated peptide in the lipid bilayer as depicted in fig.3b.

In conclusion, the present study clearly indicates that fatty acid acylation is necessary for membrane

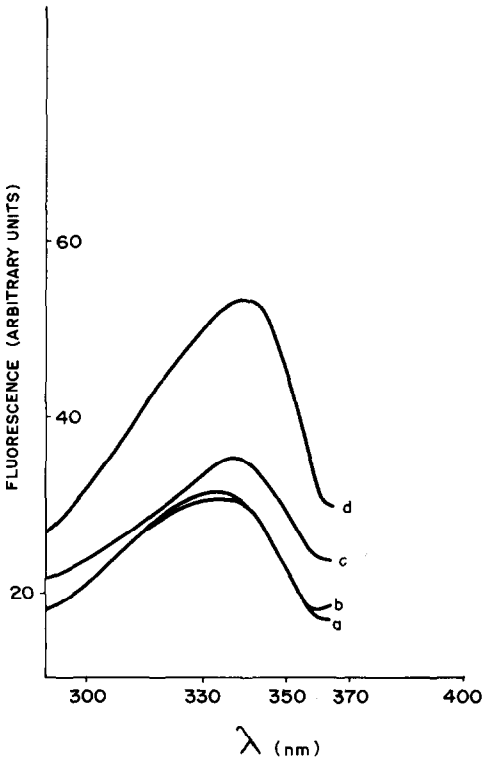

Fig.2. Fluorescence emission spectra of peptides 1 and 2 in the presence of lipid vesicles as a function of time. [Peptide] = 6 μM, [lipid] = 250 μM. (a) 1 + lipid vesicles, immediately after addition; (b) 2 + lipid vesicles, immediately after addition; (c) 1 + lipid vesicles, after 12 h; (d) 2 + lipid vesicles, after 12 h.

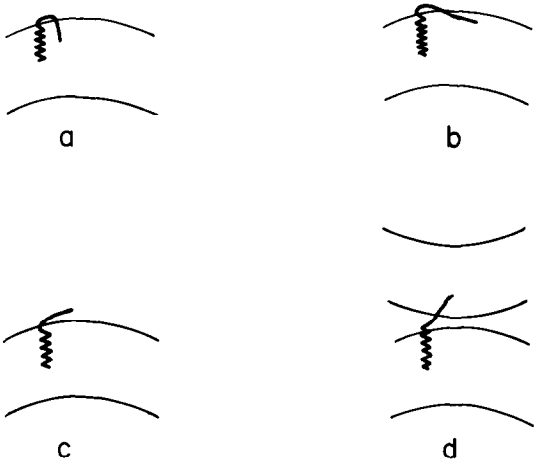

Fig.3. Schematic representation of the possible modes of association of fatty acylated peptide, Lys-(ϵ-palmitoyl)-Phe-Phe-Phe-Ile-Ile-OCH_3, with lipid vesicles: ≋ , fatty acid chain; ∫ , peptide chain.

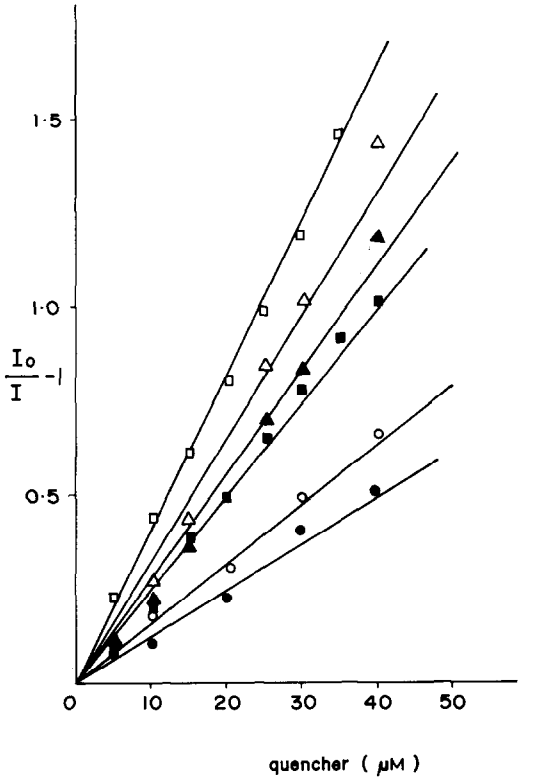

Fig.4. Stern-Volmer plots for quenching of fluorophores in peptides 1–3 and 12-(9-anthroyloxy)stearate by 5- and 16-doxylstearates: [peptide] = 6 μM, [lipid] = 250 μM. Quenching by 16-doxylstearate: (△) peptide 3, (□) 12-(9-anthroyloxy)stearate, (○) peptide 2; quenching by 5-doxylstearate: (▲) peptide 3, (■) 12-(9-anthroyloxy)stearate, (●) peptide 2.

association of even a fairly hydrophobic peptide. We are currently investigating the interaction of long hydrophobic peptides covalently modified with fatty acids of different chain lengths at different positions along the peptide chain in order to gain further insight into the role of fatty acylation in the assembly of membrane proteins.

REFERENCES

[1] Von Heijne, G. (1981) Eur. J. Biochem. 120, 275–278.
[2] Rapport, T.A. and Weidmann, M. (1985) Curr. Top. Membranes Transp. 24, 1–63.
[3] Davis, G.N., Boeke, J.D. and Model, P. (1985) J. Mol. Biol. 181, 111–121.
[4] Davis, G.N. and Model, P. (1985) Cell 41, 607–614.

[5] Ponechynsky, M.S., Tyndall, C., Both, G.W., Sato, F., Bellamy, A.R. and Atkinson, P.H. (1985) J. Cell Biol. 101, 2199–2209.
[6] Zerial, M., Huylenbroeck, D. and Garoff, H. (1987) Cell, 147–155.
[7] Von Heijne, G. and Bloemberg, C. (1979) Eur. J. Biochem. 97, 175–181.
[8] Engelman, D.M. and Steitz, T.A. (1981) Cell 23, 411–422.
[9] Magee, A.I. and Schlesinger, M.J. (1982) Biochim. Biophys. Acta 694, 279–289.
[10] Sefton, B.M. and Buss, J.E. (1987) J. Cell Biol. 104, 1449–1453.
[11] Rose, A.K., Welch, W.J., Sefton, B.M., Esch, F.S. and Ling, N.C. (1980) Proc. Natl. Acad. Sci. USA 77, 3884–3888.
[12] Joseph, M. and Nagaraj, R. (1987) Biochim. Biophys. Acta 911, 231–237.
[13] El Amin, B., Anantharamaiah, G.M., Royer, G.P. and Means, G.E. (1979) J. Org. Chem. 44, 3442–3444.