Differential penetration of fatty acyl-coenzyme A and fatty acylcarnitines into phospholipid monolayers

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Received 17 November 1994

Abstract The ability of fatty acyl-CoA's and fatty acylcarnitines to penetrate phospholipid monolayers was comparatively studied, in view of the important role of both kinds of derivatives in fatty acid transport across mitochondrial membranes. The interaction occurs predominantly through hydrophobic forces. Acylcarnitines penetrate phospholipid monolayers more strongly than acyl-CoAs; in addition the former show a positive cooperativity when they bind to the interface. These properties would facilitate membrane transfer of fatty acylcarnitines over that of their CoA homologues.

Key words: Fatty acyl-coenzyme A; Fatty acylcarnitine; Phospholipid monolayer; Model membrane

1. Introduction

Coenzyme A- and carnitine-derivatives of fatty acids are important intermediates in lipid metabolism. In particular, transfer of fatty acyl residues from cytosol into the mitochondrial matrix, where fatty acid β-oxidation occurs, requires an enzyme-catalyzed exchange of coenzymes, so that fatty acylcarnitines, but not fatty acyl-CoAs, traverse the inner mitochondrial membrane. According to standard biochemistry textbooks [1,2] coenzyme exchange is required because cell membranes are impermeable to fatty acyl-CoAs, yet biophysical studies on this point are, to the authors' knowledge, unavailable.

As a part of a research project on the comparative interactions of fatty acyl-CoAs and fatty acylcarnitines with model biomembranes, a first step has been made by studying the penetration of both kinds of fatty acyl derivatives into phospholipid monolayers, through changes in the surface pressure. Studies in the Langmuir trough are ideal for understanding amphiphile–membrane interactions, since phospholipid monolayers constitute simple models allowing direct observation of intermolecular interactions, and yet these measurements can be safely transferred to the physiological situation in which a lipid bilayer must be considered [3–5]. In monolayer experiments at constant area, the increase in surface pressure upon addition of an amphiphile to the subphase is interpreted in terms of physical penetration of at least part of the amphiphile into the film [6–11].

In our studies of acyl-CoA and acylcarnitine penetration, the hydrocarbon chain length of both phospholipids and fatty acyl derivatives has been changed in order to study the hydrophobic components of the interaction; the polar group of phospholipids has also been changed in order to identify electrostatic interactions. Fatty acylcarnitines appear to interact positively with the lipid monolayer, unlike their homologous coenzyme A derivatives.

2. Materials and methods

Acyl-CoAs and acylcarnitines were supplied by Sigma Chemical Co. (Milwaukee, WI). Acyl-CoAs were ≥ 92–95% pure, according to the supplier, and acyl carnitines were ≥ 98% pure; the purities were checked by thin-layer chromatography and, for acyl-CoAs, also by spectrophotometry. The impurities of fatty acyl-CoAs consisted of free fatty acid and free coenzyme A, as shown by UV absorption and thin-layer chromatography [12]. Egg-yolk phosphatidylcholine (EYPC) was grade I from Lipid Products (South Nutfield, England). Dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidyl-ethanolamine (DPPE), dimyristoyl-phosphatidylcholine (DMPC), dimyristoylphosphatidic acid (DMPA), bovine heart cardiolipin and L-α-phosphatidyl-DL-glycerol (Na salt) were supplied by Avanti Polar Lipids (Birmingham, AL). NaCl (Sigma) was heated at 400–500°C for 4–5 h before use in order to eliminate organic impurities. Piperazine-*N*-*N*′-bis (2-ethane-sulphonic acid) (PIPES) (Sigma) was checked in the Langmuir trough for absence of surface-active impurities. Water was double-distilled on $KMnO_4$ in a glass apparatus. The organic solvents were double-distilled before use.

The study of surface properties of monomolecular lipid layers at the air-water interface was carried our using an equipment essentially as described by Maggio et al. [13]. A Teflon trough, 20 ml in volume and 16 cm^2 in area, with magnetic stirring, was used. Surface pressure measurements were performed with an LM600 Beckman electronic microbalance. When required, the phospholipids (in chloroform solution) were spread on the air–water interface with a microsyringe. Once the phospholipid monolayer had been compressed to the desired initial surface pressure, the fatty acyl derivatives (in less than 50 μl dimethylsulphoxide) were injected into the subphase. Unless otherwise stated, fatty acyl derivative concentration in the subphase was 20 μM. In most cases experiments were carried out at a constant surface area, and at 25°C; changes in surface pressure ($\Delta\pi$) under those conditions were recorded. Average values of at least duplicate experiments are given. Non-linear fitting of $\Delta\pi$ vs. concentration curves was performed with GraFit software version 2.

3. Results and discussion

The surface-active properties of the fatty acyl derivatives under study were first tested in the absence of phospholipid. Both palmitoyl-CoA (Pa-CoA) and palmitoylcarnitine (PaCar) partition at the air–water interface with a maximum $\Delta\pi$ of 34–35 $mN \cdot m^{-1}$ (Fig. 1A). The $\Delta\pi$ vs. concentration plots can

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Abbreviations: $\Delta\pi$, increase in surface pressure; CoA, coenzyme A; DMPA, dimyristoyl phosphatidic acid; DMPC, dimyristoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DPPE, dipalmitoyl phosphatidylethanolamine; EYPC, egg-yolk phosphatidylcholine; Pa-CoA, palmitoyl-coenzyme A; PaCar, palmitoylcarnitine; PC, phosphatidylcholine.

SSDI 0014-5793(94)01326-8

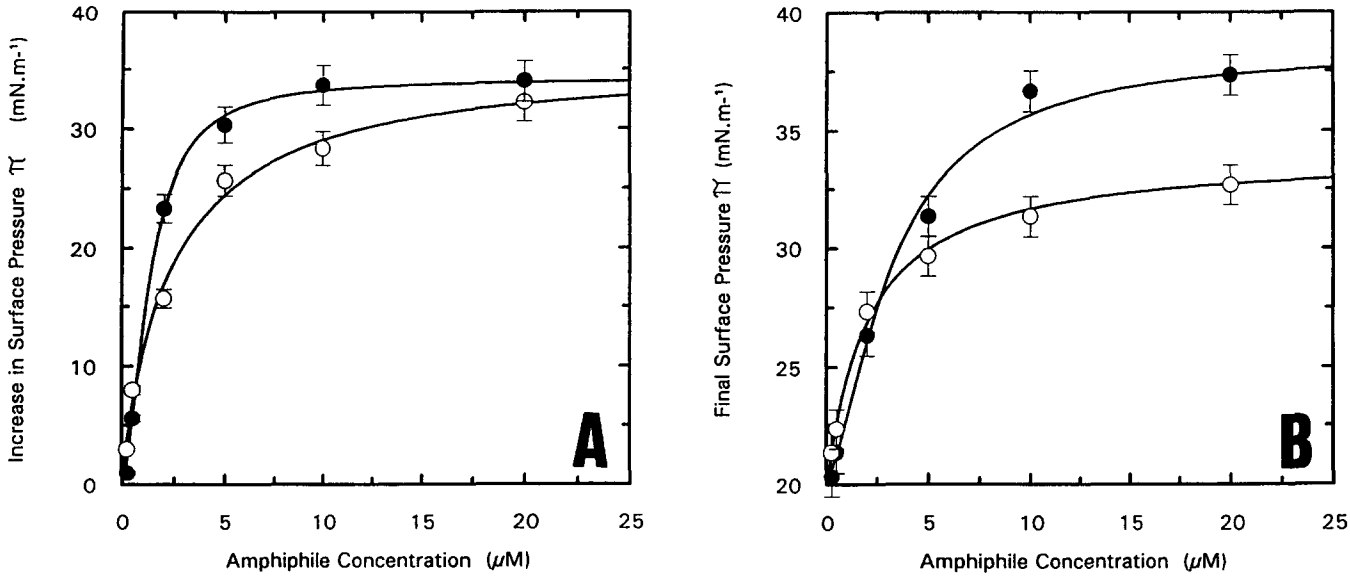

Fig. 1. Changes in the surface pressure on addition of amphiphiles to the aqueous solution in a Langmuir balance. (○) Palmitoyl-coenzyme A; (●) palmitoylcarnitine. In the absence (A) and the presence (B) of an egg-yolk phosphatidylcholine monolayer at an initial surface pressure of 20 mN·m^{-1}.

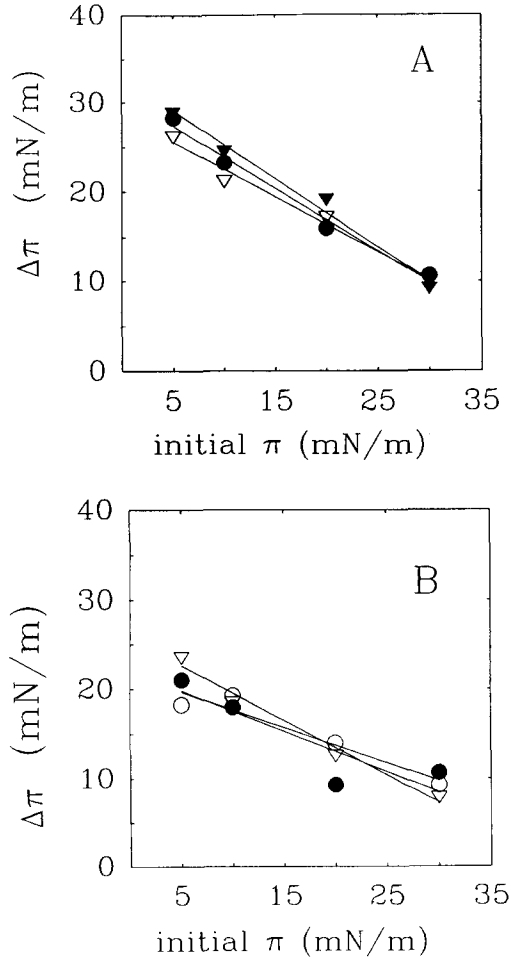

Fig. 2. Increase in surface pressure after injection of amphiphiles underneath monomolecular layers of phosphatidylcholines of different fatty acid composition. Amphiphiles were (A) palmitoylcarnitine and (B) palmitoyl-CoA. Phospholipids: (●) EYPC, (▽) DPPC, and (▼) DMPC. Final amphiphile concentration in the subphase was 20 μM.

be analyzed in terms of a modified form of the Langmuir isotherm:

$$\Delta\pi = [L]^n \cdot \Delta\pi_{max} / (K_d + [L])^n$$

where $[L]$ is the amphiphile concentration in the subphase, n is a cooperativity coefficient, and K_d is the apparent dissociation constant (an apparent K_d is obtained since we do not know the exact amount of adsorbed molecules, but only a degree of binding y, such that $y = \Delta\pi/\Delta\pi_{max}$). When this analysis is applied, $K_d = 2.2\ \mu M$ and $n \approx 1$ is found for Pa-CoA, while PaCar has $K_d = 1.6\ \mu M$ and $n = 1.7$. Thus Pa-CoA partitions at the interface in a simple, hyperbolic way, while PaCar shows a degree of cooperativity (or pseudocooperativity).

When similar measurements are carried out in the presence of a pre-formed EYPC monolayer at 20 mN·m^{-1}, in order to detect any intermolecular interactions (Fig. 1B), again Pa-CoA has a value of $n \approx 1$ while PaCar shows some sigmoidicity ($n = 1.6$) but, in addition, Pa-CoA has $K_d = 2.0\ \mu M$ and a final surface pressure π_{final} ($\Delta\pi_{max} + \pi_{initial}$) = 34 mN·m^{-1}, while PaCar has $K_d = 6.3\ \mu M$ and $\pi_{final} = 38.5$ mN·m^{-1}. Thus PaCar penetrates to 4–5 mN·m^{-1} above the adsorption equilibrium pressure (in the absence of lipids), suggesting an increase in its lateral stability due to its interaction with EYPC; a larger surface pressure implies a smaller surface free energy [11]. In contrast, Pa-CoA adsorbs to the interface in the same way either in the presence of in the absence of phospholipids.

In order to detect any specific effect of the hydrophobic moieties on the interaction between phospholipid monolayers and fatty acyl derivatives, a series of experiments were performed in which monolayer composition and chain length of fatty acyl-CoA and fatty acylcarnitines were independently varied. In each case, the increase in surface pressure was recorded after addition of 20 μM fatty acyl derivative to the subphase, at different initial surface pressures. With carnitine derivatives, the equilibrium penetration was reached in 5–10 min, while CoA derivatives took about 25–30 min to reach equilibrium.

Fig. 2 shows, for various phosphatidylcholines, the increase

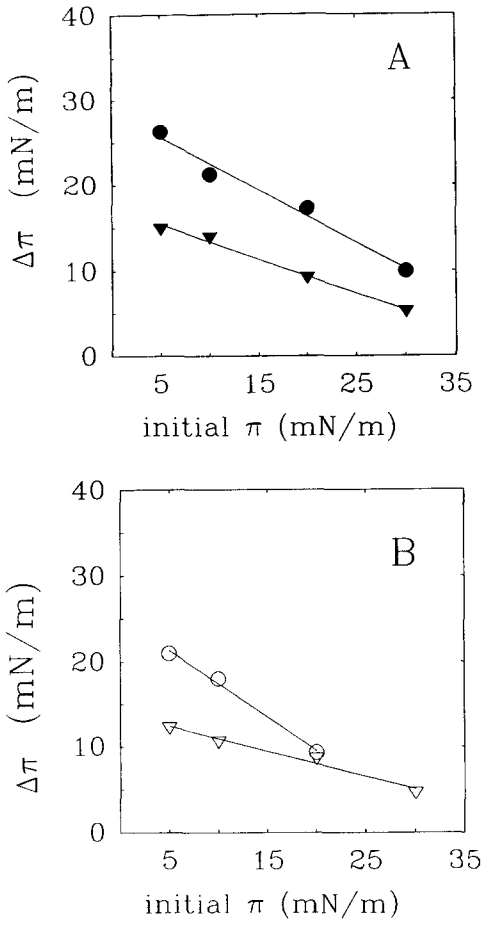

Fig. 3. Increase in surface pressure after injection of amphiphiles of different fatty acyl chain length underneath monolayers of EYPC. Amphiphiles were (A) palmitoylcarnitine and (B) palmitoyl-CoA. Fatty acyl chains: (circles) C16; (triangles) C14.

in surface pressure produced by PaCar (Fig. 2A) and Pa-CoA (Fig. 2B) at different initial pressures. In agreement with the previously discussed results, PaCar interacts more strongly than Pa-CoA with PC monolayers. In addition, Fig. 2 shows that phospholipid fatty acyl chain length or unsaturation does not significantly influence monolayer penetration by the amphiphiles under study. The limiting cut-off pressure, i.e. the pressure at which penetration no longer occurs, so that $\Delta\pi = 0$, estimated from extrapolation of the experimental data, is about 43–45 $mN \cdot m^{-1}$ for PaCar, and 49–56 $mN \cdot m^{-1}$ for Pa-CoA.

The effect of amphiphile chain length was explored with monolayers composed of phosphatidylcholines, the interaction of which with C14 and C16 and carnitine-CoA derivatives was measured. As a representative example, the results with EYPC are shown in Fig. 3. Similar results are obtained for DMPC and DPPC (not shown). For both carnitine and CoA derivatives, the interaction with EYPC is stronger for the longer fatty acyl chain, suggesting a significant role for hydrophobic interactions in the penetration process. Boylan and Hamilton [14] already observed, in an acyl-CoA/PC vesicle system, that the binding of acyl-CoA to PC bilayers was dependent on the acyl chain length.

In turn, the type of polar headgroup of the phospholipid does not appear to have a significant influence on the monolayer penetration by PaCar or Pa-CoA. Fig. 4 shows the increase in surface pressure induced by those two amphiphiles in monolayers composed of a variety of neutral and charged phospholipids. No effect is detected that can be attributed to changes in the affinity for a particular phospholipid headgroup.

In conclusion, the interaction of fatty acyl-CoAs and fatty acylcarnitines with phospholipid monolayers is mainly governed by hydrophobic forces; in particular, the fatty acyl chain length of the amphiphiles appears to be of significance. Palmitoyl-carnitine differs qualitatively and quantitatively from palmitoyl-CoA in its interaction with monolayers: the former amphiphile, but not the latter, interacts positively with phospholipids. In addition, palmitoylcarnitine penetrates the monolayer leading to mixtures with smaller surface free energies than palmitoyl-CoA. The above results provide a physicochemical basis for the coenzyme A–carnitine exchange that occurs on the cytoplasmic side of the inner mitochondrial membrane during fatty acyl import. The positive interaction of fatty acylcarnitines, but not of fatty acyl-CoAs, with phospholipids, translated to the physiological situation of the fatty acyl-derivatives in the inner mitochondrial membrane, means that fatty acylcarnitines will be 'anchored' to the membrane hydrophobic matrix more strongly, i.e. with a higher affinity, than the fatty acyl-CoAs [5]. In turn, the increased affinity of fatty acylcarnitines for the bilayer will ensure their availability as substrates for diffusion, either simple or facilitated, across the membrane, and prevent them from diffusing back to the cytoplasmic aqueous phase. The opposite process, i.e. conversion of fatty acylcarnitines into fatty acyl-CoAs, and for the opposite reasons, occurs on the matrix side.

Acknowledgments: M.A.R. is a Pre-doctoral Fellow of the Basque Government and M.G. is a Scholar of CONICOR (Córdoba, Argentina). This work was supported in part by funds from DGICYT (Grant PB91/0441) the Basque Government (Grant PGV9242), CONICOR and CONICET. G.F. thanks the Basque Government for a Visiting Fellowship.

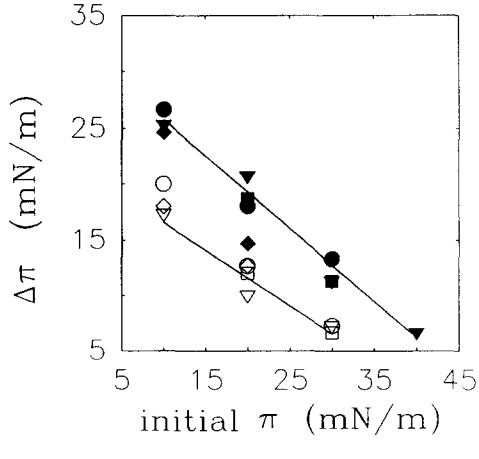

Fig. 4. Increase in surface pressure after injection of amphiphiles underneath monomolecular layers of phospholipids. Amphiphiles were palmitoyl carnitine (filled symbols) and palmitoyl-CoA (open symbols). Phospholipids: EYPC (circles); DPPC (diamonds); phosphatidyl-glycerol (triangles); cardiolipin (squares).

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