ELSEVIER

Biochimica et Biophysica Acta 1420 (1999) 95–103

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Permeation of dicarboxylic acids with different terminal position of two carboxylic groups through planar bilayer lipid membranes

Veronika Yu. Evtodienko [a], Dmitry I. Bondarenko [b], Yuri N. Antonenko [a,*]

[a] *A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russian Federation*
[b] *A.N. Bakh Institute of Biochemistry, Russian Academy of Science, Moscow 117071, Russian Federation*

Received 26 March 1999; accepted 27 May 1999

Abstract

Electrically silent hydrogen ion fluxes across a planar bilayer lipid membrane (BLM) induced by an addition of dicarboxylic (DC) acids at one side of BLM are monitored by measuring pH changes in the unstirred layers near the BLM surface via recording protonophore-dependent potentials. Two groups of DC acids are studied: (1) 2-*n*-alkylmalonic acids with an alkyl chain of different length which carry both carboxylic groups at one terminus of the hydrocarbon chain (α,α-DC acids); and (2) dicarboxylic acids of different linear chain length having carboxylic groups at the opposite ends of the hydrocarbon chain (α,ω-DC acids). It is shown that the pH optimum of hydrogen ion fluxes for the DC acids is shifted considerably to acidic pH values compared to monocarboxylic acids and is located near pH 5. For both types of DC acids at $pH \ll 5$, the total transport is limited by diffusion of the anionic forms of the acids across the unstirred layers, while at $pH \gg 5$ the transport is limited by diffusion of the neutral form across the membrane. The fluxes of α,α-DC acids are similar to those of α,ω-DC acids provided that the acids have the similar number of carbon atoms, the fluxes grow with the increase in the chain length of the alkyl radical.

Keywords: Dicarboxylic acid; Bilayer lipid membrane; Permeability; Unstirred layer; pH gradient

1. Introduction

The process of the permeation of fatty acids (FA) and their derivatives through membranes has attracted much attention during the last years due to its involvement in the mechanism of uncoupling of mitochondria [1,2]. It has been shown, in particular, that only FA derivatives which are able to 'flip-flop' in a protonated form across the membrane can activate the protein-mediated H^+-transport in proteoliposomes [3]. However, the studies conducted by different groups have led to controversial conclusions about the ability of different FA to permeate through the bilayer lipid membranes (BLMs). Namely, it has been shown in [4,5] that FA can diffuse rapidly across membranes of liposomes with time constants of tens of s^{-1}. On the other hand, it has been concluded that the time constant of FA flip-flop is much lower [6,7] (does not exceed 0.01 s^{-1} [6]). The studies of the electrogenic fluxes of hydrogen ions induced

Abbreviations: TTFB, a protonophore tetrachlorotrifluoromethylbenzeimidasole; MES, 2-[*N*-morpholino]ethanesulfonic acid; BLM, bilayer lipid membrane; FA, fatty acid; DC, dicarboxylic acid; α,α-DC11, 2-*n*-octylmalonic acid; α,α-DC14, 2-*n*-undecylmalonic acid; α,ω-DC10, sebacic acid; α,ω-DC12, dodecanedioic acid; USL, unstirred layer; DPhPC, diphytanoylphosphatidylcholine

* Corresponding author. Fax: +70-95-939-3181;
E-mail: antonen@libro.genebee.msu.su

0005-2736/99/$ – see front matter
PII: S0005-2736(99)00095-4

by FA also have not given unambiguous results. Although Gutknecht observed the increase in the BLM electrical conductance by lauric and some other acids [8], it is unlikely that this process contributes to H^+-conductance of natural membranes [1,9,10].

Dicarboxylic acids having carboxylic groups at the opposite ends of the hydrocarbon chain (α,ω-DC acids) represent an interesting class of FA derivatives with bactericidal properties [11–13]. It has been shown that most of these acids unable to rapidly flip-flop across liposome membranes [14], whereas they can diffuse through the lipid part of the inner mitochondrial membrane [12]. It is suggested in [14] that the inability to exert a fast flip-flop is associated with adopting a specific conformation in the membrane interior, possibly a U-shape conformation which may be dependent on the membrane lipid composition. On the other hand, dicarboxylic acids are known to interact with proteins [15,16]. In particular, 2-*n*-alkylmalonates were used successfully to probe the active center of the mitochondrial dicarboxylate transporter [17].

In our previous works, we studied the permeation of weak acids and bases through a planar bilayer lipid membrane by measurements of steady-state pH shifts in the unstirred layers (USLs) near the BLM [18–20]. The method showed its reliability since the estimated values of membrane permeabilities were consistent with those measured by other techniques [21]. In particular, the study of the permeation of monocarboxylic acids of different chain length enabled us to identify different limiting steps of the transport process under various experimental conditions and to estimate the membrane permeability for several acids [20]. In the present work, we have applied this technique to study the permeation of dicarboxylic acids belonging to two different groups: (1) 2-*n*-alkylmalonic acids with an alkyl chain of different length which carry both carboxylic groups at one terminus of the hydrocarbon chain ($RCH(COOH)_2$; α,α-DC acids); and (2) dicarboxylic acids of different linear chain length having carboxylic groups at the opposite ends of the hydrocarbon chain ($HOOC-(CH_2)_n-COOH$; α,ω-DC acids). It has been shown, in particular, that in our system the fluxes of α,α-DC acids across the membrane are similar to those of α,ω-DC acids provided that the acids have similar number of carbon atoms.

2. Materials and methods

BLMs were formed by a conventional method [22] in a hole, 0.4 mm in diameter, of a diaphragm dividing a PTFE chamber. The membrane-forming solution contained 20 mg diphytanoyl phosphatidylcholine (Avanti Polar Lipids) in 1 ml of *n*-decane (Merck). Electrical resistance of the BLM was measured with the help of OES-2 patch-clamp amplifier (Opus, Moscow). pH gradients on the BLM (ΔpHs) were measured according to [18,23] by the method of recordings an open-circuit potential in the presence of a protonophore (10 μM tetrachlorotrifluoromethylbenzeimidasole, TTFB) which was added at both sides of the BLM. The potential had plus sign on the side of the membrane where the acid was added. The experiments were carried out at room temperature (21–23°C).

J_{H^+} values were estimated from the following equation [20]:

$$J_{H^+} = \frac{D_{buff} \cdot \Delta pH \cdot B}{2 \cdot \delta}$$

where D_{buff} is a diffusion coefficient of the buffer molecules, B is the buffer capacity of the solutions, ΔpH is the pH gradient on the BLM, δ is the thickness of the USL. We used the value of the D_{buff} of 5×10^{-6} cm^2/s and δ of 200 μm following [19]. ΔpHs were calculated from the values of protonophore-dependent potentials according to Nernst equation [18].

pH shifts in the unstirred layers near the BLM were measured directly by means of an antimony pH microelectrode according to [20]. It should be noted that a sum of two pH shifts at the opposite sides of BLM is equal to ΔpH gradient on BLM. It has been shown previously that the two methods give consistent results [23]. Typically the electrode tip was about 10 μm in our experiments. Smooth approach of the microelectrode to the membrane was carried out by means of a hydraulic microdrive.

2-*n*-Alkylmalonic acids were prepared by alkylating of diethyl malonate with suitable *n*-alkyl halides. The distilled diethyl 2-*n*-alkylmalonates were hydrolyzed, and the products were purified by crystallization from organic solvents. The absorption maximum of the products was observed at 213–214 nm. Their molar extinction indexes depended slightly on the alkyl residue length, increasing from 137 (methyl)

to 159 M^{-1} cm^{-1} (dodecyl). Melting points of malonic acids corresponded to the data published. Synthesized 2-*n*-alkylmalonic acids contained 97–99.8% of basic compounds according to gas chromatography of their dimethyl esters. Sebacic and dodecanedioic acids were from Fluka. Tris, MES, β-alanine, KCl, and choline chloride were from Serva; TTFB was a gift of Prof. E.A. Liberman.

3. Results

In the present work, pH gradients on the BLM were induced by dicarboxylic acids of two groups: 2-*n*-alkylmalonates (α,α-DC acids), and α,ω-DC acids. Experiments with measurements of the electrical resistance of the BLM have shown that under our experimental conditions, the DC acids used in this study do not affect this parameter of the membrane implying that the proton fluxes induced by DC acids across the BLM are electrically silent. In fact, the typical values of the H^+ fluxes in our experiments were of the order of 10^{-11} mol/cm^2/s. These values greatly exceed the upper limit of the electrogenic fluxes, about 2×10^{-15} mol H^+/cm^2/s, estimated from the level of the BLM conductance (less than 10 nS/cm^2) according to the equation [24]:

$$J_{H^+} = \frac{RTt_{H^+}G_m}{z_{H^+}^2F^2}$$

where G_m is the membrane conductance, t_{H^+} is the transference number for H^+ (1.0 in our estimation), R is the gas constant, T is the absolute temperature, z_{H^+} is the ionic valence, and F is the Faraday constant.

Fig. 1 shows a typical curve of the generation of the pH gradient on the BLM induced by an addition of α,α-DC11 (octylmalonic) acid at one side of the membrane. A steady-state value of the pH gradient is reached in several minutes similarly to the case of other weak acids [18]. Fig. 1B shows the dependence of the pH gradient on the length of the hydrocarbon chain for α,α-DC acids. pH gradients induced by α,α-DC5 and α,α-DC7 acids are close to zero. On the other hand, the solubility of the acids decreases dramatically upon the lengthening of the hydrocarbon chain, especially in neutral and acidic pH ranges. Thus, only several DC acids are suitable for the experimental study in a wide range of pH, namely α,α-DC11 (pH ≥ 3.2) and α,α-DC14 (pH ≥ 6) acids (having 11 and 14 carbon atoms in molecules, respectively) and α,ω-DC10- (pH ≥ 4) and α,ω-DC12 (pH ≥ 6) acids (having 10 and 12 carbon atoms in molecules, respectively).

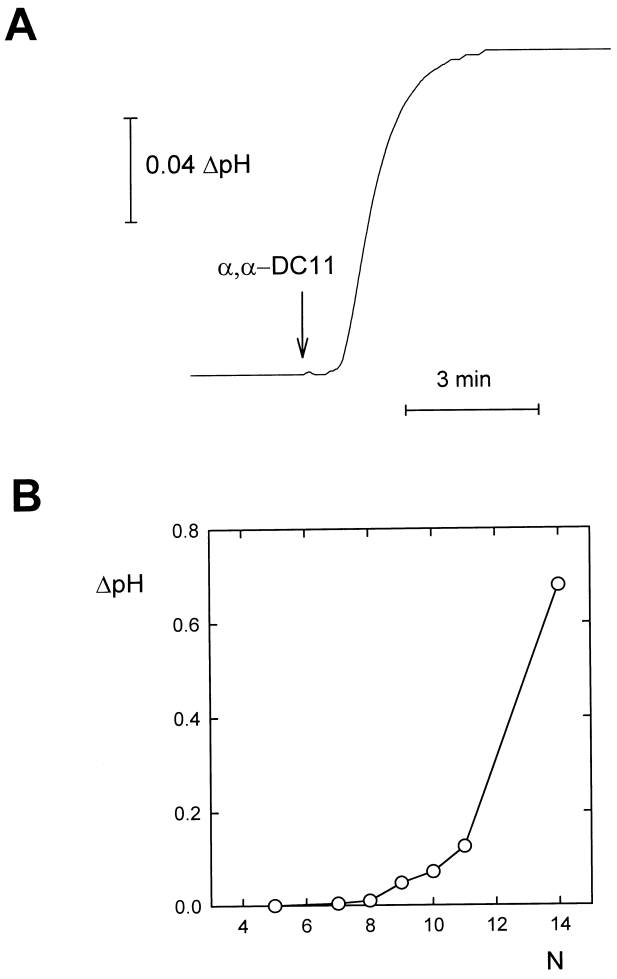

Fig. 1. (A) An example of the measurement of the pH gradient on the BLM induced by 0.2 mM α,α-DC11 acid (octylmalonic acid). The medium was: 1 mM Tris, 1 mM MES, 1 mM β-alanine, 100 mM choline chloride, pH 5.0. (B) The effect of the number of carbon atoms in the 2-*n*-alkylmalonic acids (*N*) on the pH gradient on the BLM (ΔpH) generated under the conditions used in A. The data for $N = 14$ were calculated from the measurements at pH 6.0 since this acid is poorly soluble at pH 5.0.

It is worth noting that the establishment of a steady-state value of protonophore-dependent potential several minutes after the addition of the DC acid (Fig. 1) indicates that the pH profiles in the USLs and the transmembrane proton flux reach their

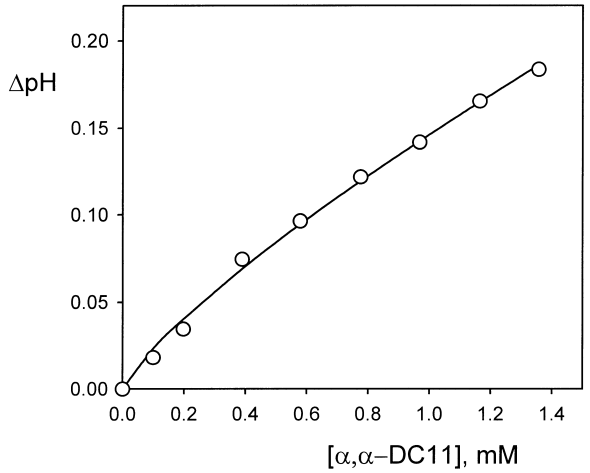

Fig. 2. The dependence of the pH gradient on the BLM (ΔpH) on the concentration of α,α-DC11 at one side of the membrane. The medium was as in the caption to Fig. 1, but pH was 6.0.

steady-state values. The value of the flux does not decrease within the time scale of our experiments (tens of minutes), since in contrast to the flux measurements on liposomes, our system is characterized by a very high ratio of the bulk volume to the flux which prevents the increase in the DC acid concentration in the *trans* compartment of our experimental cell.

It has been shown previously that the concentration dependence of the pH gradients in the USLs is sublinear due to the inhibiting effect of high pH gradients on the transmembrane H^+-ions flux induced by weak acids [20,25,26]. As seen from Fig. 2, this effect is also characteristic of DC acids.

To confirm the idea that the potentials measured in the presence of a protonophore correspond to the pH gradients on the BLM, we measured pH shifts in the unstirred layers near the membrane directly by the pH microelectrode. Fig. 3 shows the pH profile in the USL near the BLM induced by the addition of α,α-DC11 at the same (*cis*) side of the BLM. The amplitude of the pH profile amounts to 0.066 under these conditions which is about half of the total pH gradient on the BLM estimated from the protonophore-dependent potential measurements (0.12) since the total pH gradient on the BLM corresponds to a sum of two pH shifts at the opposite sides of the membrane.

pH dependencies of the pH gradients (ΔpHs) induced by DC acids are presented in Fig. 4. The dependencies for α,α-DC11 (Fig. 4, curve 1) and α,ω-DC10 (Fig. 4, curve 2) have sharp maxima at pH 5. Thus, pH_{max}, i.e. the pH values at which the maximum pH gradient is observed, for DC acids shifts considerably with respect to that for monocarboxylic acids for which it is about pH 7 [20]. The maximum values of hydrogen ion fluxes $(J_{H^+})_{max}$ induced by DC acids (estimated as it is described in Section 2) are close to $(J_{H^+})_{max}$ of monocarboxylic acids having the same number of carbon atoms ($CH_3(CH_2)_8COOH$, 34×10^{-12} mol $H^+/cm^2/s$ and α,ω-DC10 24×10^{-12} mol $H^+/cm^2/s$). As it was mentioned above, it is impossible to measure the pH dependence for DC acids with a longer chain due to their limited solubility at $pH < 6$.

It has been shown previously that the pH dependence of ΔpH induced by monocarboxylic acids can be

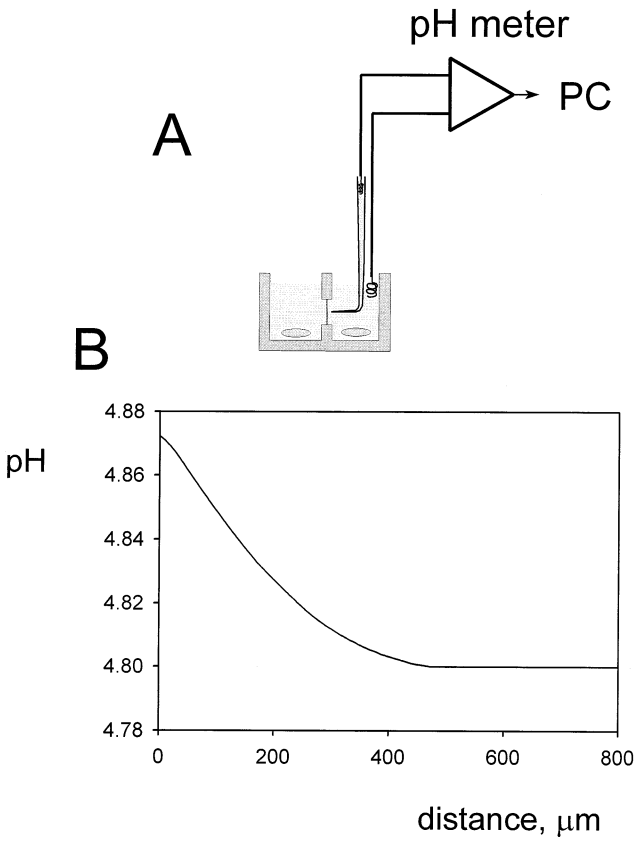

Fig. 3. (A) A scheme of the pH profile measurements by a pH microelectrode. (B) pH profile in the *cis* unstirred layer near the BLM induced by the addition of 0.2 mM α,α-DC11 at the *cis* side of the BLM. The medium was as in the caption to Fig. 1, pH was 4.8.

accounted for by the change of the limiting stage of the total transport from that occurring in the USLs to that proceeding in the BLM itself [20]. The test revealing these two cases was based on the effect of the intensity of the solution stirring on the thickness of the USLs, δ [20,27]. More intensive solution stirring is expected to reduce the BLM potential when the transport is limited by the stage occurring in the membrane; on the contrary, the stirring should have no effect on the potential when the limiting stage of the transport proceeds in the USL.

Fig. 5 shows the recordings of the BLM potential induced by α,α-DC11 acid at low (pH 3.6, right side) and high (pH 6.0, left side) pH values. In agreement with the results of [27], the increase in the rate of the solution stirring reduces the value of the potential at neutral pH and does not affect the BLM potential in the acidic pH range.

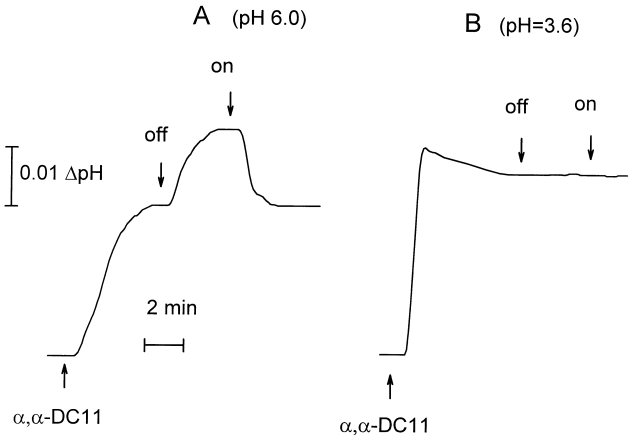

Fig. 5. Effect of the stirring on the value of the pH gradient on the BLM induced by α,α-DC11 (the addition made at one side of the BLM is marked by an arrow). (A) 1.5 mM of the acid, pH 6.0. (B) 0.5 mM of the acid, pH 3.6. The medium was: 10 mM citrate, 100 mM choline chloride. At the moments marked by arrows (off and on) the medium stirring was switched off and on, respectively, on both sides of the membrane.

4. Discussion

The present work deals with the electrically silent H^+-fluxes across the BLM induced by DC acids. It can be concluded from the measurements of the BLM electrical conductance that the acids studied in the present work can permeate through the membrane in the neutral form only (TH_2) and the membrane is impermeable for anionic forms of the acids (T^{2-} and TH^-). All three forms are in an equilibrium in the solution:

$$T^{2-} + H^+ \leftrightarrow TH^-$$

$$TH^- + H^+ \leftrightarrow TH_2$$

with pK_{a1} and pK_{a2}, respectively. Since TH_2 is the only membrane-permeable form, proton-consuming reactions predominate in the USL at the *cis* side of the BLM (where the acid is added), whereas proton-liberating reactions predominate at the opposite side of the BLM (Fig. 6). Thus, transmembrane pH gradients are formed in the USLs.

A quantitative description of the process of the permeation of the DC acid through the BLM including the formation of local pH gradients on the membrane is rather complex and leads to equations which have only the numerical solution [19]. However, one can use a simplified model of weak acid permeation (as it was proposed in our previous paper [20]) and derive analytical solutions using several simplifications. The main one is the assumption that the pH shifts in the USLs are small. Supposing that the

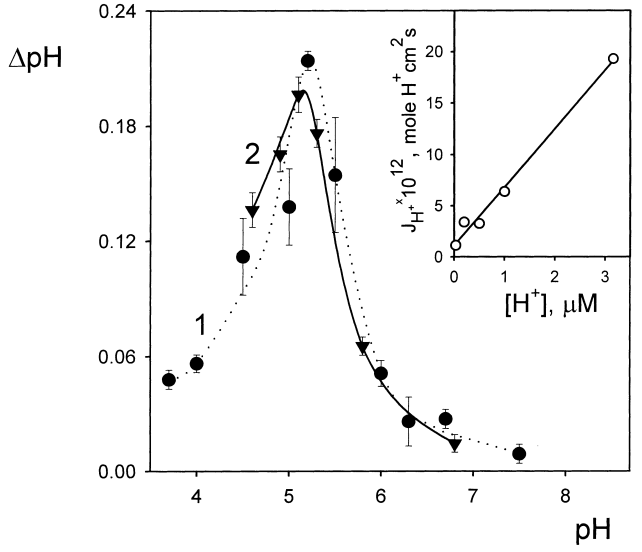

Fig. 4. pH dependence of the pH gradient on BLM (ΔpH) induced by the addition of 0.2 mM α,α-DC11 (curve 1) or 0.2 mM α,ω-DC10 (curve 2) at one side of the BLM. The solution was as in the caption to Fig. 1. Each point is a mean ± S.E. of 4–8 independent experiments. Inset: the dependence of the hydrogen ion flux (J_{H^+}) induced by the addition of 0.2 mM α,α-DC11 at one side of the BLM on the hydrogen ion concentration in the bulk phases.

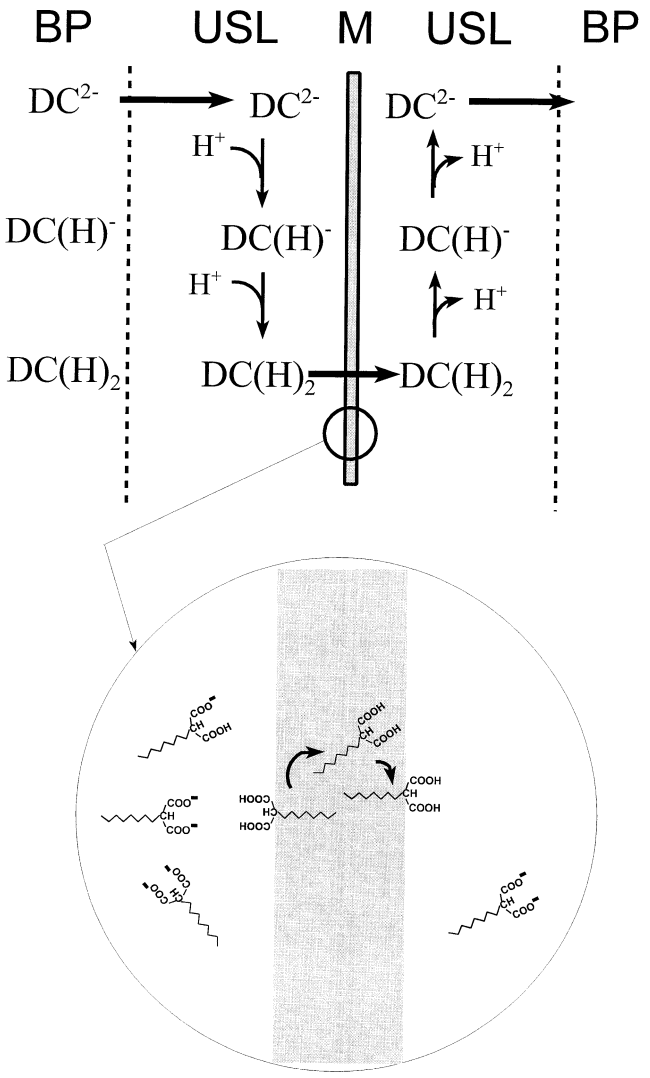

Fig. 6. A scheme of the permeation of a dicarboxylic acid (DC) through unstirred layers (USL) and a bilayer lipid membrane (M) accompanied by proton transfer reactions in the unstirred layers. The arrows show the proton route leading to the formation of the pH gradient on the membrane. BP, bulk phase.

chemical reactions are fast compared to transmembrane permeation and that the USL permeabilities of all three forms of the DC acid are the same (P^{USL}), one can derive the following equation describing the dependence of the H^+-flux on the pH of the solutions (modified Eq. 5 of [20]):

$$J_{H^+} = \frac{[T_0]P^{M}_{TH_2}(2\alpha_1\alpha_2 + \alpha_1)}{(\alpha_1\alpha_2 + \alpha_1 + 1)(\alpha_1\alpha_2 + \alpha_1 + 1 + P^{M}_{TH_2}/P^{USL})} \quad (1)$$

where $P^{M}_{TH_2}$ is the membrane permeability of the TH_2, P^{USL} is the USL permeabilities of TH_2, TH^- and T^{2-}, $[T_0]$ is a total concentration of the DC acid added, $\alpha_1 = 10^{pH-pK_{a1}} = K_{a1}/[H^+]$ $\alpha_2 = 10^{pH-pK_{a2}} = K_{a2}/[H^+]$. In the limiting case of alkaline pH, Eq. 1 is reduced to:

$$J_{H^+} = \frac{2[T_0]P^{M}_{TH_2}[H^+]^2}{K_{a1}K_{a2}} \quad (2)$$

while in the limiting case of acidic pH,

$$J_{H^+} = \frac{[T_0]P^{USL}K_{a1}}{[H^+]} \quad (3)$$

The analysis of the Eq. 1 shows that at $pH \ll pH_{max}$, the limiting step of the H^+-flux is the diffusion of charged forms of the acid across the USLs. A reduction of J_{H^+} with decreasing pH is caused by the reduction of T^- and T^{2-} concentration. It is worth noting that the flux of the DC acid itself is expected not to decrease, but rather to reach a constant level in this pH region. The H^+-flux decreases in this pH region because it constitutes only a part of the total DC acid flux which is designated by arrows in Fig. 6. At $pH \gg pH_{max}$ the transport is limited by the TH_2 permeation across the membrane itself and the decrease in J_{H^+} with increasing pH in the alkaline pH range is associated with the reduction of the TH_2 concentration. Thus, the model implies that the bell-shape pH dependence of H^+-flux is due to the change of the limiting step of the total process of the DC acid transport.

In order to estimate the membrane permeabilities of DC acids one should know their pK values (Eq. 1). The pK_{a1} and pK_{a2} of α,ω-DC10 and α,α-DC11 are available in the literature: 4.6 and 5.6, and 3.0 and 6.2, respectively [28]. The pK values of α,α-DC acids and α,ω-DC of different molecular weights vary insignificantly [29]. Fig. 7 shows the theoretical pH dependencies of J_{H^+} calculated as the best-fit of the experimental curves (Fig. 4) using Eq. 1. The following pK_{a1} and pK_{a2} values of DC acids were used: 3.0 and 5.8 for α,α-DC11 acid, and 4.4 and 5.6 for α,ω-DC10. Two parameters were varied: $P^{M}_{TH_2}$ and P^{USL}. It is seen from Fig. 7B, curve 1 that in the case of α,ω-DC10 the theoretical dependence agrees reasonably well with the experimental one. On the other hand, the theoretical dependence for α,α-DC11 acid deviates from the experimental

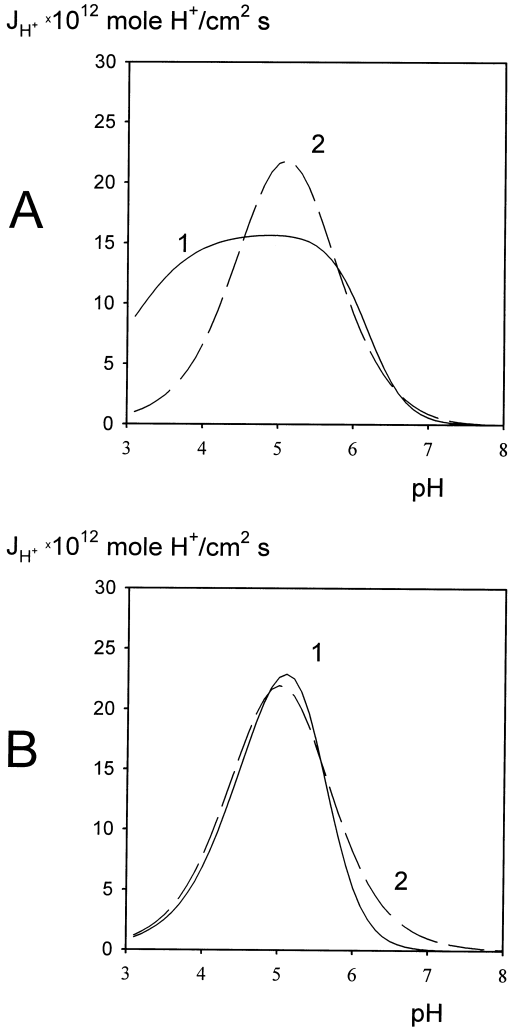

Fig. 7. The best fit of experimental J_{H^+} fluxes calculated according to Eq. 1 for α,α-DC11 (A) and α,ω-DC10 acid (B). Experimental J_{H^+} fluxes were calculated from the data of Fig. 3 as it was described in Fig. 2. Theoretical curves were obtained using the following parameters: $pK_{a1} = 3.0$, $pK_{a2} = 6.2$, $P^{M}_{TH_2} = 118 \times 10^{-3}$ cm/s, $P^{USL} = 0.08 \times 10^{-3}$ cm/s for curve 1, A; $pK_{a1} = 4.76$, $pK_{a2} = 7$, $P^{M}_{TH_2} = 1.05 \times 10^{-3}$ cm/s, $P^{USL} = 0.3 \times 10^{-3}$ cm/s for curve 2, A; $pK_{a1} = 4.6$, $pK_{a2} = 5.6$, $P^{M}_{TH_2} = 1.5 \times 10^{-3}$ cm/s, $P^{USL} = 0.19 \times 10^{-3}$ cm/s for curve 1, B; and $pK_{a1} = 4.68$, $pK_{a2} = 7$, $P^{M}_{TH_2} = 1.09 \times 10^{-3}$ cm/s, $P^{USL} = 0.3 \times 10^{-3}$ cm/s for curve 2, B.

one especially in the acidic pH range (Fig. 7A, curve 1).

Eq. 2 shows that at $pH \gg pH_{max}$ J_{H^+} is proportional to the square of the hydrogen ion concentration. The experimentally measured fluxes, however, depend linearly on the H^+ concentration in the pH region from 5.5 to 7 (Fig. 4, insert). The analysis of Eq. 1 has shown that the linear dependence of J_{H^+} on $[H^+]$ is possible in the range of $pK_{a1} < pH < pK_{a2}$ provided that the pK_{a2} value is shifted considerably to the alkaline pH range. Fig. 7, curves 2 present the best fit calculated under the conditions of $pK_{a2} = 7$ and $P^{USL} = 0.3 \times 10^{-3}$ mol/cm^2/s. This value of P^{USL} should be close to 0.3×10^{-3} mol/cm^2/s since the P^{USL} value is determined by the thickness of the USL and the diffusion coefficient which can be estimated independently [20]. In this case, we vary pK_{a1} and $P^{M}_{TH_2}$. It is seen that the theoretical curves 2 fit the experimental curves better than curves 1 especially in the case of α,α-DC11 (Fig. 7A). The calculated pK_{a1} are 4.76 and 4.68 for α,α-DC11 acid and α,ω-DC10, respectively (Fig. 7). It should be mentioned that the best-fit theoretical curves are not sensitive to variation of the pK_{a2} values if $pK_{a2} > 7$ because the experimental J_{H^+} fluxes are close to zero at $pH > 7$.

This consideration indicates that the values of the pK_a of DC acids are shifted to the alkaline pH. It can be proposed that the proton-transfer reactions involved in transmembrane permeation of the TH_2 form proceed near the surface of the BLM, and the effective value of pK_a, which determines the transport process, differs significantly from the bulk phase values in agreement with the data on the other weak acids [30–32]. The main reasons of the pK shifts at the membrane surface is the change in the hydrogen ion concentrations near the interface (bound DC acids can create a considerable negative surface potential) on the one hand, and the change in the dielectric permittivity at the interface, on the other.

One of the most important characteristics of the transport of weak acids is their membrane permeability $P^{M}_{TH_2}$. This parameter can be estimated using Eq. 2 and the J_{H^+} values in the alkaline part of the experimental pH dependence where the transport is limited by the permeation of the TH_2 form through the membrane. The $P^{M}_{TH_2}$ values were estimated using two sets of pK_a: (1) bulk phase pK_a values (curves 1 in Fig. 7); and (2) assuming pK_{a2} being more than 7 and taking pK_{a1} as the best fit of the experimental fluxes (curves 2 in Fig. 7). For the first set of pK_a values in the case of α,α-DC11 acid the following values of $P^{M}_{TH_2}$ were obtained: 41×10^{-3} cm/s (pH 6.0), 210×10^{-3} cm/s (pH 6.7), 1950×10^{-3} cm/s (pH 7.5). On the other hand, if the second set of

pK_a values was used, the calculated $P^M_{TH_2}$ value varied insignificantly with pH and was about 1×10^{-3} cm/s for this acid. The pH independence of the calculated $P^M_{TH_2}$ values indicates that the second set of pK_a values is more consistent with the experimental data. The same value of $P^M_{TH_2}$ was estimated for α,ω-DC10 (1×10^{-3} cm/s).

However, these estimations of are $P^M_{TH_2}$ based on many assumptions and can hardly be considered as unambiguously determined values. On the other hand, the $P^M_{TH_2}$ values are commonly used for estimation of fluxes of substances across membranes. Since the J_{H^+} values were proportional to the fluxes of the TH_2 form of a particular DC acid (Eq. 2) in the alkaline pH range with respect to the maximum in the pH dependence, these values can be used for the comparison of the fluxes of different DC acids. According to Fig. 4, the fluxes of α,α-DC11 are similar to those of α,ω-DC10 acid. Taking the fluxes of these DC acids as unity, we obtain the following series of flux values for α,α-DC14, α,ω-DC12, α,α-DC11, α,ω-DC10 from the experimental J_{H^+} fluxes: 12, 5, 1, 1, respectively. It can be concluded that the fluxes of α,α-DC acids and α,ω-DC acids are similar to each other, if they contain similar number of carbon atoms. Besides, the fluxes of DC acids increase with increasing the number of carbon atoms in their molecules.

The similarity of the permeabilities of α,α-DC acids and α,ω-DC acids in our system can be of considerable interest since it has been shown in [14] that liposome membranes made of a mixture of lipids are poorly permeable to dodecanedioic acid compared to, for example, lauric acid. On the other hand, this acid readily permeates through the inner mitochondria membranes [12]. The measurements of pH dependence of J_{H^+} enabled us to compare the permeabilities of these two types of acids. It has been shown in our previous work [20] that the maximum value of J_{H^+} of an acid is independent of pK_a of the acid and is determined by the ration of the membrane and the USL permeabilities. Therefore it can be used for the comparison of the membrane permeabilities of mono- and di-carboxylic acids. Comparing $(J_{H^+})_{max}$ of α,ω-DC10 (24×10^{-12} mol $H^+/cm^2/s$) and $(J_{H^+})_{max}$ of $CH_3(CH_2)_8COOH$) (34×10^{-12} mol $H^+/cm^2/s$), one can conclude that the permeabilities of mono- and di-carboxylic acids differ insignificantly under our experimental conditions, provided that they have the same number of carbon atoms. The discrepancies in the data on the permeation of α,ω-DC acids in various systems can be a result of the differences in their membrane compositions. Our experiments have shown that in the case of the BLM made of natural mixture of phospholipids (azolectin), the flux of α,ω-DC10 increased two times compared to DPhPC BLMs (data not shown). Besides, the difference in the transport of dodecanedioic acid and lauric acid observed in [14] can be due to a sharp pH dependence of the dodecanedioic acid permeation and the reduction of the transport at pH 8 which was chosen in the experiments.

Acknowledgements

The authors are grateful to Prof. V.P. Skulachev and to Dr. K.F. Sholz for helpful discussions. The work was partially supported by Grants 97-04-49703 and 96-15-97042 of the Russian Fund for Basic Research.

References

[1] V.P. Skulachev, FEBS Lett. 294 (1991) 158–162.
[2] K.D. Garlid, D.E. Orosz, M. Modriansky, S. Vassanelli, P. Jezek, J. Biol. Chem. 271 (1996) 2615–2620.
[3] P. Jezek, M. Modriansky, K.D. Garlid, FEBS Lett. 408 (1997) 166–170.
[4] F. Kamp, D. Zakim, F.L. Zhang, N. Noy, J.A. Hamilton, Biochemistry 34 (1995) 11928–11937.
[5] J.A. Hamilton, J. Lipid Res. 39 (1998) 467–481.
[6] A.M. Kleinfeld, P. Chu, J. Storch, Biochemistry 36 (1997) 5702–5711.
[7] D.D. Stump, R.M. Nunes, D. Sorrentino, L.M. Isola, P.D. Berk, J. Hepatol. 16 (1992) 304–315.
[8] J. Gutknecht, J. Membr. Biol. 106 (1988) 83–93.
[9] B. Fuks, F. Homble, Plant Physiol. 112 (1996) 759–766.
[10] A.Y. Andreyev, T.O. Bondareva, V.I. Dedukhova, E.N. Mokhova, V.P. Skulachev, L.M. Tsofina, N.I. Volkov, T.V. Vygodina, Eur. J. Biochem. 182 (1989) 585–592.
[11] S. Kolvraa, N. Gregersen, Biochim. Biophys. Acta 876 (1986) 515–525.
[12] G. Liu, B. Hinch, A.D. Beavis, J. Biol. Chem. 271 (1996) 25338–25344.
[13] J. Vamecq, J.P. Draye, J. Brison, Am. J. Physiol. 256 (1989) G680–G688.

[14] P. Jezek, M. Modriansky, K.D. Garlid, FEBS Lett. 408 (1997) 161–165.
[15] R.M. Kaikaus, W.K. Chan, P.R. Ortiz de Montellano, N.M. Bass, Mol. Cell. Biochem. 123 (1993) 93–100.
[16] R.M. Kaikaus, Z. Sui, N. Lysenko, N.Y. Wu, P.R. Ortiz de Montellano, R.K. Ockner, N.M. Bass, J. Biol. Chem. 268 (1993) 26866–26871.
[17] K.F. Sholtz, D.I. Bondarenko, D.V. Mamaev, FEBS Lett. 327 (1993) 54–56.
[18] Y.N. Antonenko, L.S. Yaguzhinsky, J. Bioenerg. Biomembr. 14 (1982) 457–465.
[19] Y.N. Antonenko, G.A. Denisov, P. Pohl, Biophys. J. 64 (1993) 1701–1710.
[20] V.Y. Evtodienko, O.N. Kovbasnjuk, Y.N. Antonenko, L.S. Yaguzhinsky, Biochim. Biophys. Acta 1281 (1996) 245–251.
[21] A. Walter, J. Gutknecht, J. Membr. Biol. 77 (1984) 255–264.
[22] P. Mueller, D.O. Rudin, H.T. Tien, W.C. Wescott, J. Phys. Chem. 67 (1963) 534–535.
[23] Y.N. Antonenko, A.A. Bulychev, Biochim. Biophys. Acta 1070 (1991) 279–282.
[24] A.L. Hodgkin, Biol. Rev. 26 (1951) 339–354.
[25] Y.N. Antonenko, L.S. Yaguzhinsky, Bioelectrochem. Bioenerg. 13 (1984) 85–91.
[26] T.X. Xiang, B.D. Anderson, Pharm. Res. 10 (1993) 1654–1661.
[27] P. Pohl, E. Rosenfeld, R. Millner, Biochim. Biophys. Acta 1145 (1993) 279–283.
[28] M. Smith, Z. Phys. Chem. 25 (1898) 189–228 (data available in Beilsteins Handbook der organischen Chemie, Berlin, 1920, band 2, pp. 718, 729).
[29] Beilsteins Handbook der organischen Chemie, Berlin, 1920, band 2.
[30] M. Langner, T. Isac, S.W. Hui, Biochim. Biophys. Acta 1236 (1995) 73–80.
[31] V. Von Tscharner, G.K. Radda, Biochim. Biophys. Acta 643 (1981) 435–448.
[32] G. Cevc, Biochim. Biophys. Acta 1031 (1990) 311–382.