J Solid State Electrochem (2012) 16:2391–2397
DOI 10.1007/s10008-012-1758-8

ORIGINAL PAPER

Membrane fluidity of tetramyristoyl cardiolipin (TMCL) liposomes studied by chronoamperometric monitoring of their adhesion and spreading at the surface of a mercury electrode

Stefan Zander · Michael Hermes · Fritz Scholz · Andreas Gröning · Christiane A. Helm · Doris Vollmer · Uwe Lendeckel · Lorenz Schild

Received: 9 March 2012 / Revised: 23 April 2012 / Accepted: 23 April 2012 / Published online: 5 May 2012

Abstract Giant unilamellar liposomes of the synthetic cardiolipin 1′,3′-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol give chronoamperometric current peaks at a stationary mercury electrode. The signals are due to the adhesion and spreading of the liposomes on the hydrophobic mercury surface. The potential dependence shows a minimum of the peak frequency at the point of zero charge, a large maximum of peak frequency at about −0.2 V and a second, however, smaller maximum at −0.8 V. The electrochemical behaviour of the liposomes indicates phase transitions of the cardiolipin which could be also observed in differential scanning calorimetry.

Keywords Cardiolipin · 1′,3′-Bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol · Liposome · Chronoamperometry · Adhesion · Spreading

Dedicated to Nina Fjodorovna Zakharchuk on the occasion of her 75th birthday.

S. Zander · M. Hermes · F. Scholz (✉)
Institute of Biochemistry, University of Greifswald,
Felix-Hausdorff-Straße 4,
17487 Greifswald, Germany
e-mail: fscholz@uni-greifswald.de

A. Gröning · C. A. Helm
Institute of Physics, University of Greifswald,
Felix-Hausdorff-Str. 6,
17487 Greifswald, Germany

D. Vollmer
Max Planck Institute for Polymer Research,
Ackermannweg 10,
55128 Mainz, Germany

U. Lendeckel
Institute of Medical Biochemistry and Molecular Biology,
University Medicine Greifswald,
Fleischmannstr. 42-44,
17475 Greifswald, Germany

L. Schild
Institute of Clinical Chemistry and Laboratory Diagnostics,
Division of Pathobiochemistry,
Otto-von-Guericke University Magdeburg,
Leipziger Str. 44,
39120 Magdeburg, Germany

Introduction

Cardiolipins (CL) are a class of phospholipids which is predominantly present in the inner mitochondrial membrane. They possess a wide structural variability which is of importance for their biological function [1]. The two pK_a values of cardiolipins are such (pK_{a1}≈2.8, pK_{a2}≈7.5 and higher) that they exist under physiological conditions as mono-anions [2]. Recently, we have observed that mitochondria undergo on the surface of a mercury electrode an adhesion–spreading process [3] which resembles the adhesion–spreading processes which we have discovered when liposomes interact with a mercury electrode [4–10], and which have been also observed in case of thrombocyte vesicles [11]. In order to better understand the mitochondria experiments, and to expand our experience with the adhesion–spreading of liposomes, we have prepared unilamellar liposomes of the synthetic tetramyristoyl cardiolipin (TMCL) 1′,3′-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (Fig. 1) and studied their interaction with a static mercury electrode surface.

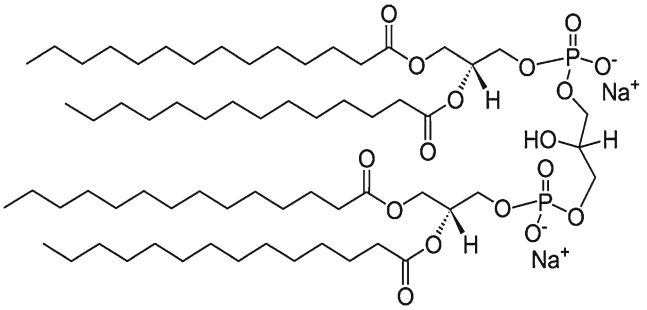

Fig. 1 Structure of the used cardiolipin 1′,3′-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (TMCL) as disodium salt (from Avanti Catalogue 2011)

Experimental

The synthetic 14:0 cardiolipin 1′,3′-bis[1,2-dimyristoyl-*sn*-glycero-3-phospho]-*sn*-glycerol (sodium salt) (other used names are: 1,1′,2,2′-tetratetradecanoyl cardiolipin (sodium salt), TMCL(1′-[14:0/14:0],3′-[14:0/14:0]), and TMCL) from Avanti Polar Lipids (USA), see Fig. 1, was purchased as chloroform solution and powder (for differential scanning calorimetric (DSC)). Unilamellar vesicles of TMCL were prepared according to a modified rapid evaporation method, initially proposed by Moscho [12]. In the electrochemical measurements, the final TMCL liposome concentration was 0.167 mg ml^{-1}, i.e., 0.13 mmol L^{-1}, and the background electrolyte was an aqueous 0.1 M KCl solution. At higher TMCL concentrations, multilamellar vesicles have been observed, which were not used for the electrochemical experiments. The chronoamperometric measurements were performed using the Autolab PGSTAT 12 (Eco Chemie, Utrecht, Netherlands) with the high-performance module ADC 750, allowing to measure fast transients with a sampling rate of 750×10^3 s^{-1}, i.e., sampling intervals down to 1.33 μs. The electrode stand VA 663 (Metrohm, Herisau, Switzerland) was used in conjunction with the Hg multimode electrode as working electrode (drop size=3), a Pt auxiliary electrode and an Ag|AgCl (3 M KCl, E=0.208 V vs. SHE) electrode. The surface area of the mercury drop was 0.48 mm^2, as determined from weighing 50 Hg drops. The adhesion–spreading of the liposomes at the Hg

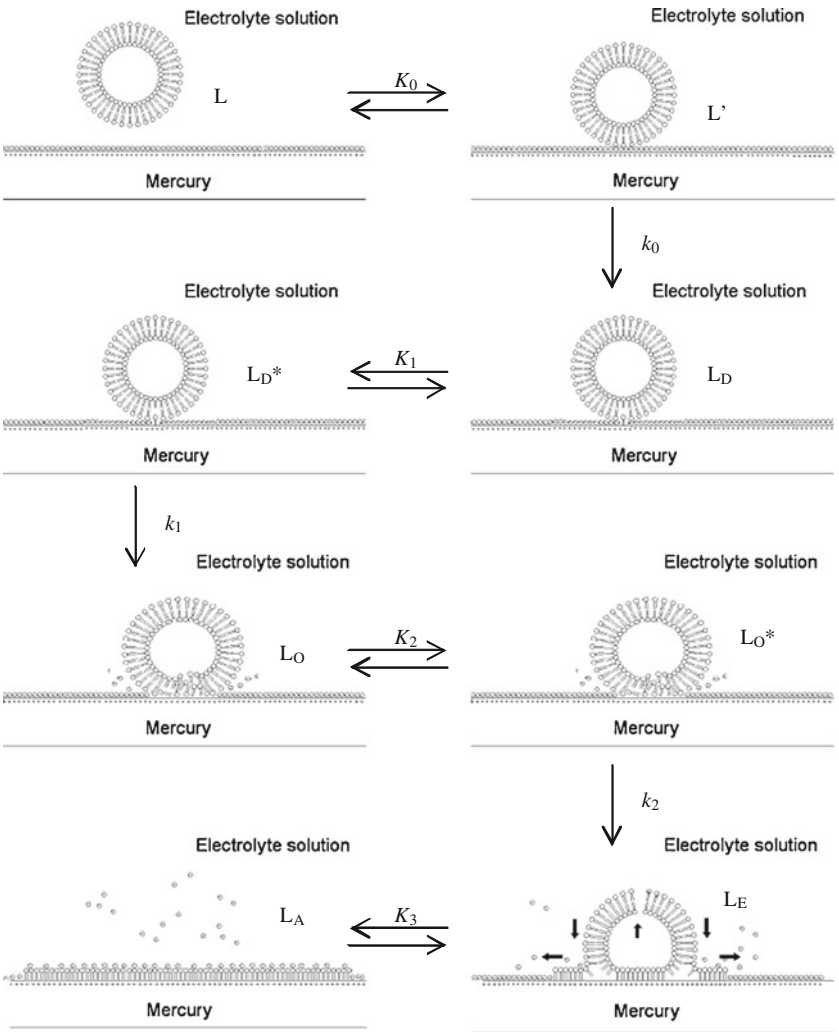

Fig. 2 Mechanism of the adhesion and spreading of liposomes on a mercury electrode (according to [5]). *L*, *L′*, etc. denote the liposomes in the different stages of their interaction with the electrode

electrode surface was followed by chronoamperometry (within 1.5 s; sampling each 50 μs (normal resolution mode) and within 0.04 s, sampling each 1.33 μs (high resolution mode)). The current range of the potentiostat was 100 nA.

The variation of the specific heat relative to a baseline was measured as function of temperature with a differential scanning microcalorimeter (VP-DSC, Microcal, Inc.). The microcalorimeter consists of coin-shaped fixed-in-place twin cells of 0.52 cm^3 each, mounted in a cylindrical adiabatic chamber [13]. The sample cell is filled with vesicles dispersed in 0.1 M KCl solution while the reference cell is filled with the same salt solution. The scan rate was 10 °C per hour. A phase transition gives rise to an endothermic (exothermic) heat change and less (more) power feedback is required to null the temperature difference between both cells. This temperature-dependent power feedback is used to calculate the specific heat $C_p(T)$ of the solution.

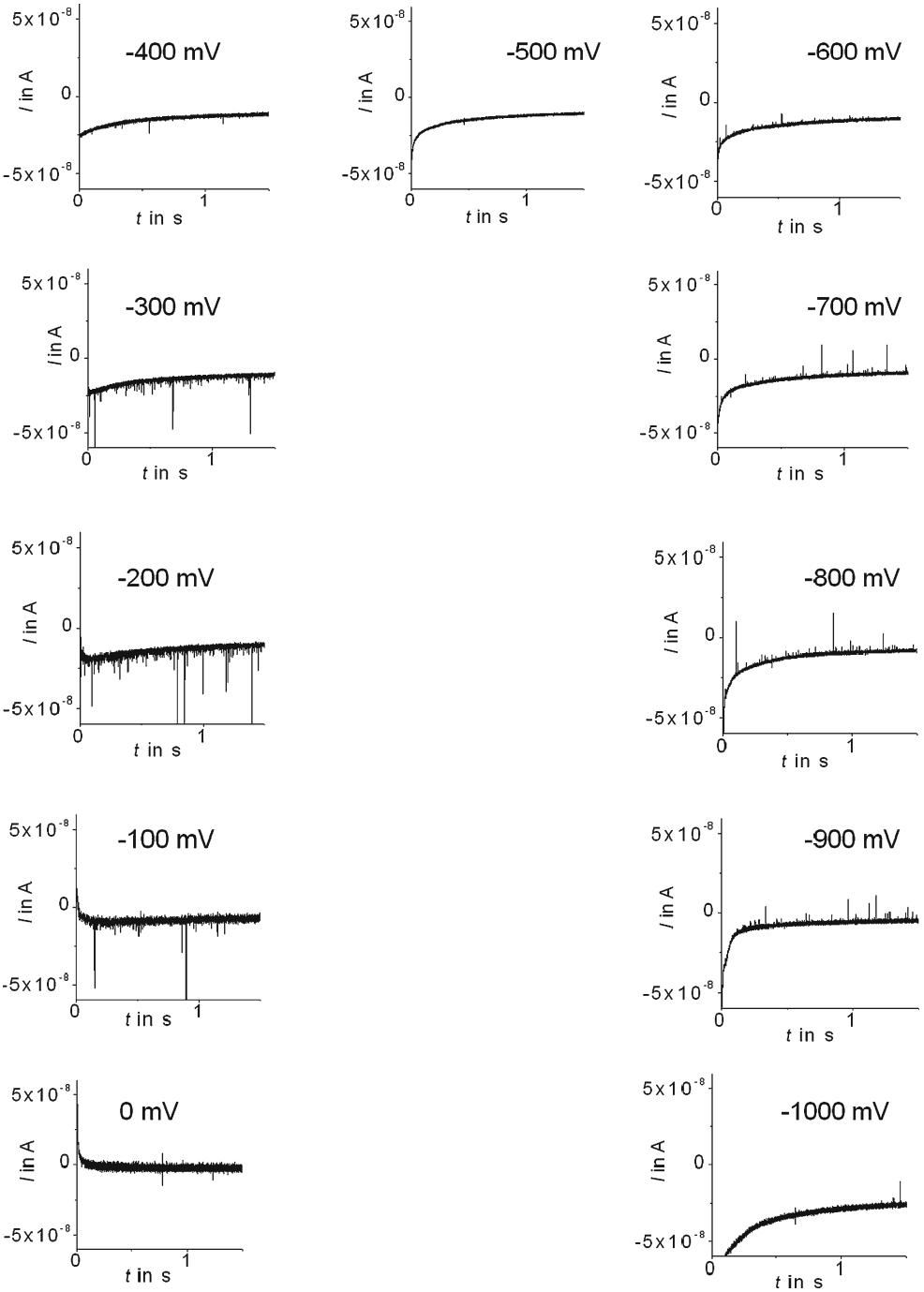

Fig. 3 Chronoamperometric traces recorded at different electrode potentials in a suspension of unilamellar TMCL liposomes in 0.1 M KCl solution at 40 °C

Results and discussion

Previous studies of the interaction of liposomes with a static mercury electrode have revealed that liposomes undergo an adhesion–spreading process as depicted in Fig. 2. The formation of islands of adsorbed lipid molecules gives rise to well-defined capacitive signals because the double-layer capacity considerably drops when the aqueous side of the double layer is substituted by a lipid monolayer. Figure 3 depicts chronoamperometric traces recorded at different electrode potentials. Each spike is due to the disintegration (adhesion–spreading) of a single liposome on the electrode surface. The spikes change their sign at the potential of zero charge (pzc), since the lipid islands formed by the adsorption and spreading of the TMCL liposomes displace water molecules and ions on the electrode surface. The mercury/solution interface is oppositely charged below and above the pzc, so that a displacement of the electrolyte layer by a dielectric layer (the CL layer) produces capacitive currents of opposite sign. The driving force for the formation of adsorbed islands of CL is the entropy gain due to the liberation of the water layer on mercury caused by the attachment of the hydrophobic tails of cardiolipins to the hydrophobic mercury surface (hydrophobic effect). The electrode potential affects the number of capacitive signals (Fig. 4) and also the height of the signals (Fig. 5). The height changes are the result of the potential dependence of the charge density of the mercury/solution interface. The smaller that charge density, the smaller the capacitive signal when TMCL molecules adsorb. The change of numbers of detected signals per time results because decreasing signals lead to a loss of *countable* signals because the smallest ones are not distinguishable from noise [5, 6]. Thus, the dependence of signals on potential is only an *apparent* one resulting from the detection technique. The charge displacement at potentials negative to the point of zero charge (around −450 mV vs. Ag/AgCl) gives rise to positive current spikes, while negative currents result from the respective process at potentials positive to the pzc (Fig. 3). Figure 4 proves that there are adhesion–spreading events even for liposomes which are negatively charged. Given the concentration of electrolyte (0.1 mol L^{-1} KCl), the Debye length of the liposomes and the Gouy length of the electrode are certainly very small so that the liposomes can approach the electrode without much repulsion. It is interesting to see that the negative charge of the liposomes does not prevent adhesion–spreading events. The anchoring of the liposomes must be caused by some TMCL molecules which have turned around in the liposome because of the flip-flop kinetics within the membrane, or, if the flip-flop kinetic is too slow, some molecules may be present in the reversed configuration as defects. Such turned around TMCL molecules can be seen as the nucleation centres for the anchoring–spreading of liposomes [8, 9]. These turned around molecules can anchor because of the very favourable release of iceberg water around the alkyl chains and from the electrode double layer when the TMCL molecule attaches to the Hg surface with its hydrophobic side. This mechanism is obviously also operative for negatively charged liposomes.

Figure 6 shows the Arrhenius plot for the *macrokinetics*, i.e., the rate of adhesion–spreading events measured as the number of peaks per units of time and surface area in the temperature range from 2 to 47 °C. For this, ln J was plotted versus the reciprocal temperature, with J being the peak frequency f divided by the product of the surface area of the mercury drop A_{SMDE} and the Avogadro constant N_{A}.

$$J = \frac{f}{A_{\mathrm{SDME}} \cdot N_{\mathrm{A}}}$$

The plot shows three distinct breaks, typically indicating phase transitions. The first break appears at 40.5 °C and it is most likely due to the transition between the lamellar liquid

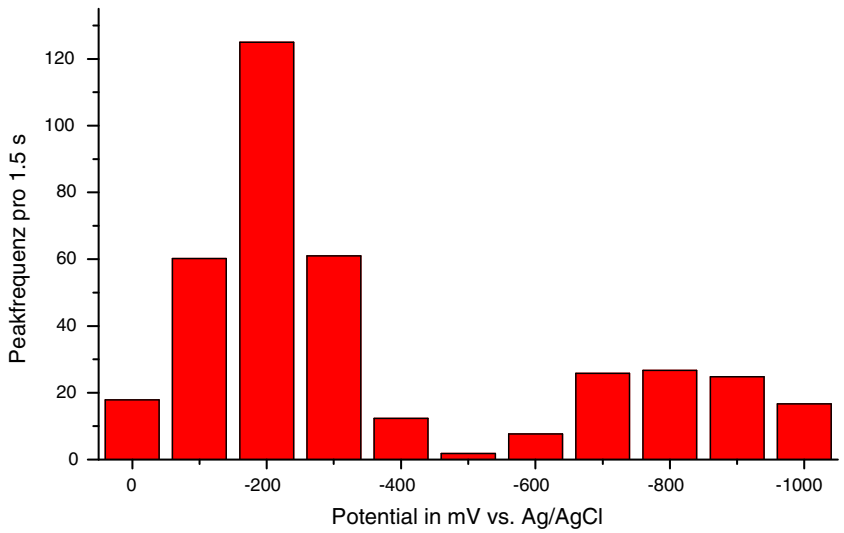

Fig. 4 Number of adhesion–spreading events (chronoamperometric traces) per time at different electrode potentials for TMCL GUV liposomes

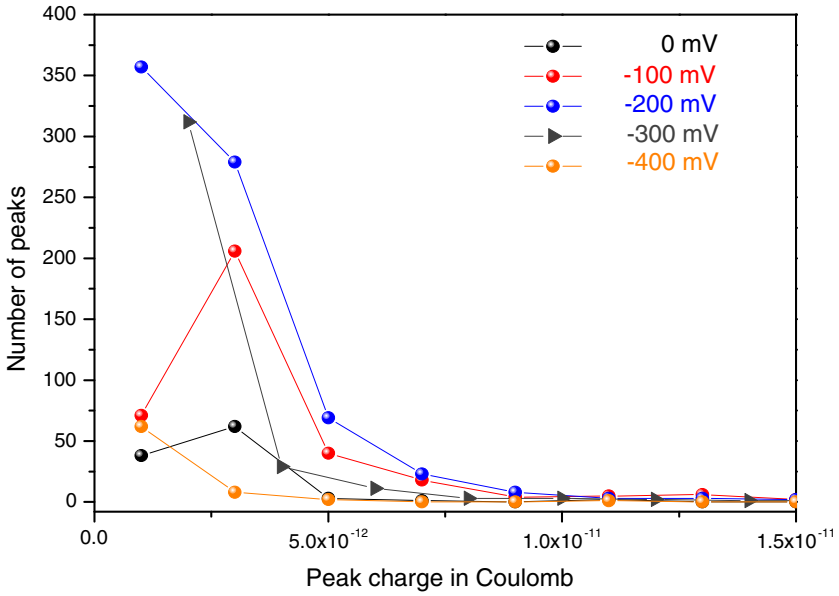

Fig. 5 Number of adhesion–spreading events and size of the respective capacitive current signals at different electrode potentials for TMCL GUV liposomes

crystalline phase and the lamellar gel phase $L_\beta \rightleftarrows L_\alpha$. The phase transitions reported in literature relate to the ammonium and sodium salts of TMCL: 40 °C [14], 33 °C, and 35 °C [15, 16] are given, respectively. Figure 6 exhibits a second-phase transition at 22.5 °C, which can be due to the transition between the lamellar gel and the subgel phase $L'_C \rightleftarrows L_\beta$. This assumption is supported by literature data giving the range 24.9–29.2 °C [17] for that transition. The discontinuity at 12.4 °C (Fig. 6) may correspond to a low-temperature endothermic transition which has been reported to occur in the range 14–18 °C [16]. The activation energies E_A derived from the slopes of the straight parts of the plot given in Fig. 6 are decreasing with increasing temperature. This is an indication of increasing fluidity of the different phases going from the low temperature to the higher temperature phases. Above 45 °C, the experimental data were much too scattered to derive reliable information. The rather sluggish transition between the lamellar gel phase and the subgel phase (22.5–30 °C) cannot be clearly explained; however, it is possible that nucleation growth is responsible for it. Liposomes are rather small entities, implying a small concentration of nuclei, of whatever nature they may be. If the formation of nuclei is slow and their number is

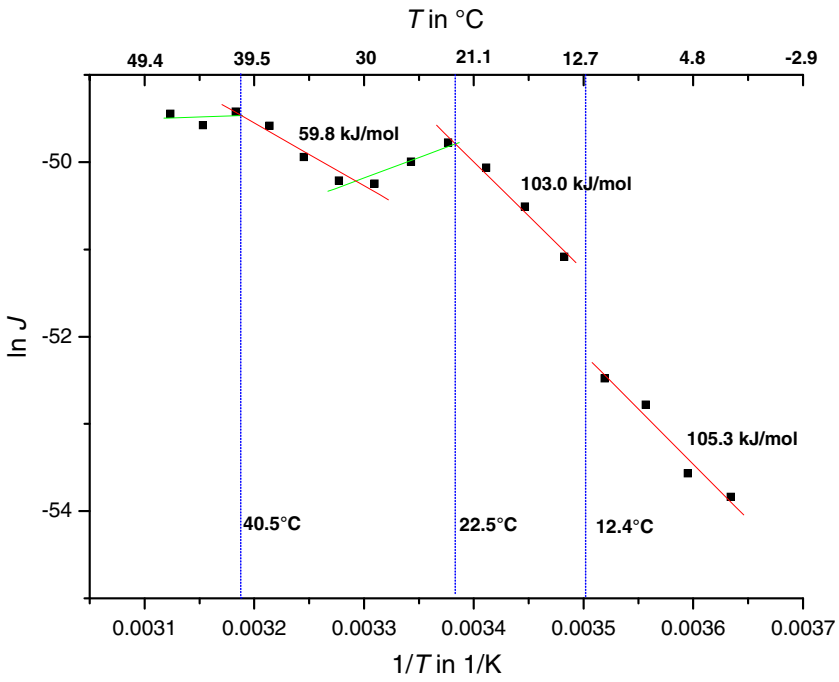

Fig. 6 Arrhenius plot for a suspension of TMCL GUV liposomes. Electrode potential: –200 mV vs. Ag/AgCl

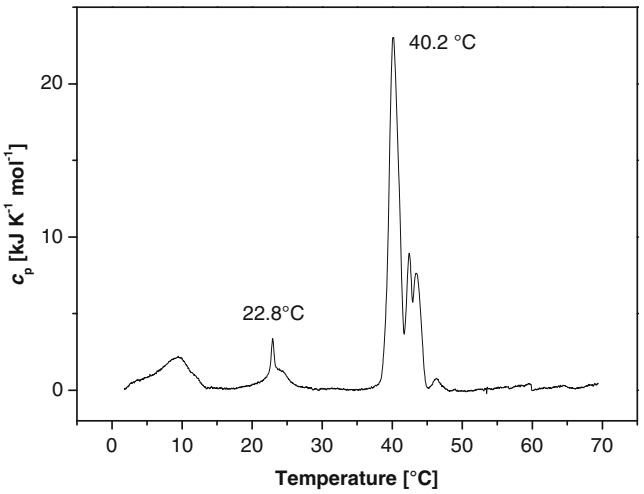

Fig. 7 Differential scanning calorimetry of a TMCL liposome suspension (0.5 mg mL^{-1} TMCL, 0.1 M KCl)

small, it is feasible that there is always a fraction of liposomes which may be even free of any nucleus, and it is unlikely that nuclei transfer occurs from one liposome to another. This all can considerably contribute to a very sluggish phase transformation of an ensemble of independent liposomes dispersed in a solution of rather low liposome concentration, as studied here.

To be able to compare the phase transition data derived from the electrochemical experiments with independently obtained data, DSC measurements were performed with TMCL liposomes. These measurements had to be performed with a higher liposome concentration (0.5 mg mL^{-1}). Nevertheless, they confirmed the gel to liquid–crystalline phase transition at 40.2 °C, and the subgel to gel phase transition at 22.8 °C, as well as a broad endothermic peak around 10 °C (cf. Fig. 7). The structured peak in the range of 39–43 °C is most probably due to the high TMCL concentration. Further, it is likely that this high concentration is also responsible for the slight increase of the transition around 20 °C. The enthalpy change of the phase transition at 40.2 °C was 57.25 kJ mol^{-1} which is in good agreement with the literature value of 52 kJ mol^{-1} [18]. The transition at 22.8 °C has an enthalpy change of 7.5 kJ mol^{-1} which is also a reasonable value for the subgel to gel transition.

The disintegration of *single* liposomes can be analysed by studying the time dependence of charge displacement $Q(t)$ during single adhesion–spreading events. This is what we call the *microkinetics* of the adhesion–spreading process. In the gel phase L_β, at a temperature of 40 °C and at potentials of −900 and −200 mV (maxima of Fig. 3), respectively, the adhesion–spreading signals (the spikes shown in Fig. 3) were fitted using the equation:

$$Q(t) = Q_0 + Q_1\left(1 - \exp\left(\frac{-t}{\tau_1}\right)\right) + Q_2\left(1 - \exp\left(\frac{-t}{\tau_2}\right)\right) \quad (1)$$

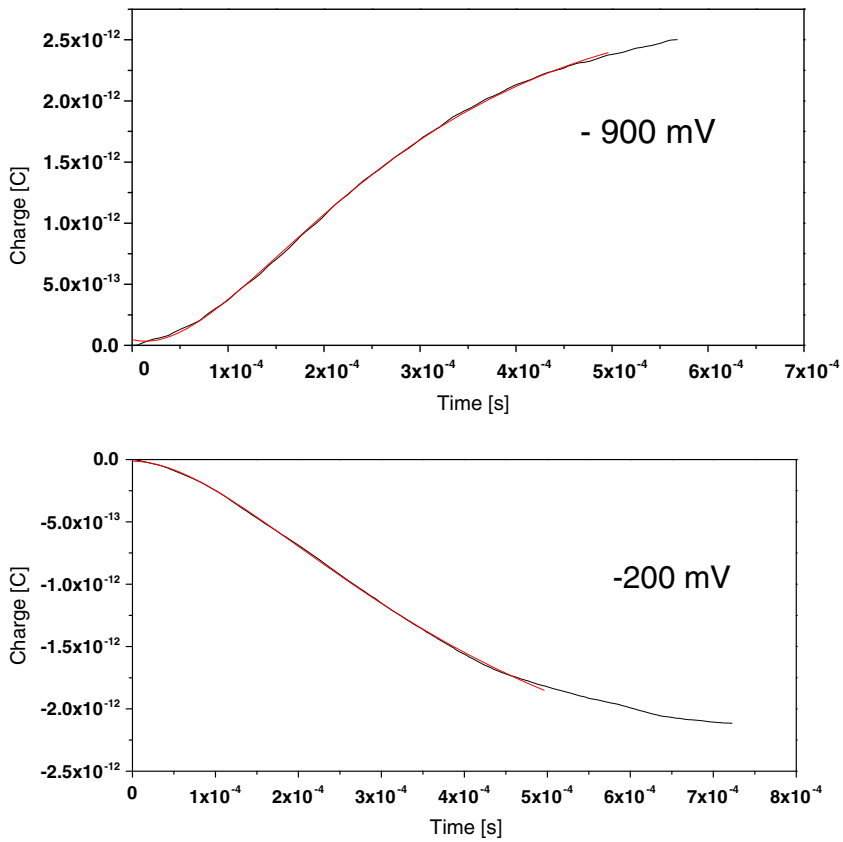

Fig. 8 Charge–time transients, resulting from the integrated current–time curve of a single adhesion–spreading event of a TMCL liposome, at the potentials −900 and −200 mV (*black* experimental and *red* fitted curve)

The time constants, τ_1 and τ_2 are connected with the thermodynamic equilibrium constants K_n of the adsorption process, respectively, as follows [5, 6]:

$$\tau_n = \frac{1 + K_n}{k_n K_n} \tag{2}$$

τ_n refers to the opening (n=1) and the spreading step (n=2), respectively. That exponential equation is the same as that used before for the adsorption–spreading process of liposomes [5] and supported also by other researchers [19]. The best fitting produced the following time constants:

1. Potential −900 mV:

$$\tau_1 = (7.3 \times 10^{-5} \pm 3.2 \times 10^{-5})\text{s}$$
$$\tau_2 = (3.7 \times 10^{-4} \pm 1.6 \times 10^{-4})\text{s}$$

2. Potential −200 mV:

$$\tau_1 = (9.4 \times 10^{-5} \pm 3.3 \times 10^{-5})\text{s}$$
$$\tau_2 = (3.4 \times 10^{-4} \pm 1.9 \times 10^{-4})\text{s}$$

The fitted experimental curves are shown in Fig. 8. The fit and the time constants show that the TMCL liposomes behave very similar to the previously studied DMPC, DOPC, and other liposomes [5–7].

Conclusions

The results show that TMCL liposomes lend themselves for adhesion–spreading studies on mercury electrodes. Such experiments provide information on the phase transition temperatures; the activation parameters of the macrokinetics can be derived, as well as the time constants of the microkinetics (and certainly also the activation parameters of the different steps of the microkinetics). The results are in agreement with the interpretation of the mitochondria experiments as the cardiolipins of the mitochondrial membrane are obviously able to contribute to their adhesion–spreading behaviour. This work paves the way to study the interaction of foreign molecules with cardiolipins along the outlined approach of studying the effects of these agents on the flexibility of the liposome membrane. Future work will be focussed on studying the effect of an oxidative attack TMCL liposomes has with respect to membrane fluidity.

Acknowledgments The authors acknowledge financial support from Phospholipid Research Center, Heidelberg, Germany, and by Deutsche Forschungsgemeinschaft (He 1616/14).

References

1. Schlame M (2008) J Lipid Res 49:1607–1620
2. Kates M, Syz JY, Gosser D, Haines TH (1993) Lipids 28:877–882
3. Hermes M, Scholz F, Härdtner C, Walther R, Schild L, Wolke C, Lendeckel U (2011) Angew Chem Int Ed 50:6872–6875
4. Hellberg D, Scholz F, Schauer F, Weitschies W (2002) Electrochem Commun 4:305–309
5. Hellberg D, Scholz F, Schubert F, Lovrić M, Omanović D, Agmo Hernández V, Thede R (2005) J Phys Chem B 109:14715–14726
6. Agmo Hernández V, Scholz F (2006) Langmuir 22:10723–10731
7. Agmo Hernández V, Scholz F (2008) Israel J Chem 48:169–184
8. Agmo Hernández V, Hermes M, Milchev A, Scholz F (2009) J Solid State Electrochem 13:639–649
9. Agmo Hernández V, Milchev A, Scholz F (2009) J Solid State Electrochem 13:1111–1114
10. Agmo Hernández V, Scholz F (2008) Bioelectrochemistry 74:149–156
11. Agmo Hernández V, Niessen J, Harnisch F, Block S, Greinacher A, Kroemer HK, Helm CA, Scholz F (2008) Bioelectrochemistry 74:210–216
12. Moscho A, Orwar O, Chiu DT, Modi BP, Zare RN (1996) PNAS USA 93:11443–11447
13. Vollmer D, Strey R, Vollmer J (1997) J Chem Phys 107:3619–3626
14. Rainier S, Jain MK, Ramirez F, Ioannou PV, Marecek JF, Wagner R (1979) Biochim Biophys Acta 558:187–198
15. Sankaram MB, Powell GL, Marsh D (1989) Biochim Biophys Acta 980:389–392
16. Lewis RNAH, Zweytick D, Pabst G, Lohner K, McElhaney RN (2007) Biophys J 92:3166–3177
17. Nagamachi E, Kariyama R, Kanemasa Y (1985) Physiol Chem Phys Med NMR 17:255–260
18. Cevc G, Marsh D (1987) Phospholipid bilayers. Physical principles and models, vol 5. Wiley, New York, p 242
19. Ruzic I, Pecar-Ilic J, Ivosevic DeNardis N (2010) J Electroanal Chem 642:120–126