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A humidity titration calorimetry technique to study the thermodynamics of hydration

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Abstract

Isothermal titration calorimetry has been applied in combination with a moisture generator and, independently, gravimetry to study the adsorption of water onto 50 μg of the lipid dioleoylphosphatidylcholine. During the experiment, the relative humidity is varied stepwise, e.g. from 2% to 92% in increments of 2% every 15 min. The difference between the partial molar enthalpy of the water molecules which interact directly with the lipid headgroups and the molar enthalpy of bulk water is endothermic (10–15 kJ/mol). We conclude that the hydration of lipids with phosphatidylcholine headgroups is entropy-driven at room temperature.

1. Introduction

Practically all biologically relevant molecules and structures require water to attain their native conformation and structure. For example, phospholipids bind considerable amounts of water at their polar headgroups, thus establishing a liquid–crystalline lamellar arrangement which can be considered as the structural basis of biological membranes. The bound water does not only promote the formation of a membrane but gives rise to a considerable repulsive force towards other hydrated (membrane) surfaces [1–3]. This so-called hydration force is a prerequisite of a stable cellular membrane because it prohibits the spontaneous attachment and fusion of membranes.

Although a basic phenomenon in biophysics, the physical principle driving the hydration of phospholipids and other bio-relevant molecules has not yet been understood in detail. On the one hand, the water can be expected to establish hydrogen bonds to certain polar moieties of the lipid headgroups and, in turn, to the previously bound water molecules. Such a preferential arrangement of water molecules close to the membrane interface constitutes an enthalpically favourable state. On the other hand, hydration can be considered as a 'lubrication' phenomenon promoting the mobility and conformational freedom of the lipid moieties. In this case, water binding would be driven by entropic forces.

Therefore, establishing the enthalpic and entropic effects of hydration can be considered a key to decide which of the phenomena discussed above plays the dominating role under certain conditions. For this purpose, we introduce a calorimetric method to determine the enthalpy of hydration independently. This 'humidity titration' technique is an

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adaptation of classic isothermal titration calorimetry to measure the heat which evolves upon adsorption of a gaseous sorbate (e.g., water) onto the sorbent (e.g., lipid). Several attempts to measure adsorption heats have been reported. Batch calorimeters work in the 'isopiestic' mode where the instrument measures the heat response after starting equilibration between the sorbent and the condensed phases of the sorbate (e.g., water) via its gas phase [4–6]. Flow calorimeters work in the perfusion mode where the sample is exposed to a stream of an inert gas which is partially saturated with the gaseous sorbate [7]. In this application, the partial pressure of the sorbate must be precisely adjusted, varied, and re-adjusted by a gas generator. Our technique is based on an insert cell placed into an adiabatic titration calorimeter and its perfusion with moist nitrogen was provided by a computer-controlled humidity generator. Although the principle is not new, we claim that the experimental approach presented here is superior in three respects: (1) the subtle, fast and precise regulation of the relative humidity (RH) of the gas; (2) the time regime of the measurement which guarantees complete equilibration at each external condition chosen; and (3) the very simple combination of the calorimeter and the gas generator. In this Letter, we give a first report about the experimental approach. It has been applied to study the thermodynamics of hydration of phospholipids. Here we present first results. A detailed study on this topic will be given elsewhere.

2. Materials and methods

2.1. Adsorption calorimetry

We used a MicroCal MCS isothermal titration calorimeter which has been supplemented with a commercial solid sample insert cell (MicroCal Inc., see Fig. 1). This device is made of stainless steel and is cylindrical in shape with ~ 2 cm height and 2 mm diameter.

Typically, 50 μg of the sample lipid (1,2-dioleoyl-sn-glycerol-3-phosphocholine, DOPC; Avanti Polar Lipids, Alabaster, AL), dissolved in an organic solvent (e.g., chloroform/methanol, 2:1 v/v), was filled into the body of the insert cell (volume ~ 50 μl). Then, the insert cell is placed into a somewhat tilted, slowly rotating vacuum evaporator to remove the solvent. This procedure precipitates the lipid as a thin film covering the inside walls of the insert cell (area ~ 1 cm^2). After complete drying, the insert cell is closed with its cover, thoroughly sealed with varnish, and introduced into the water-filled calorimeter cell through the access tube.

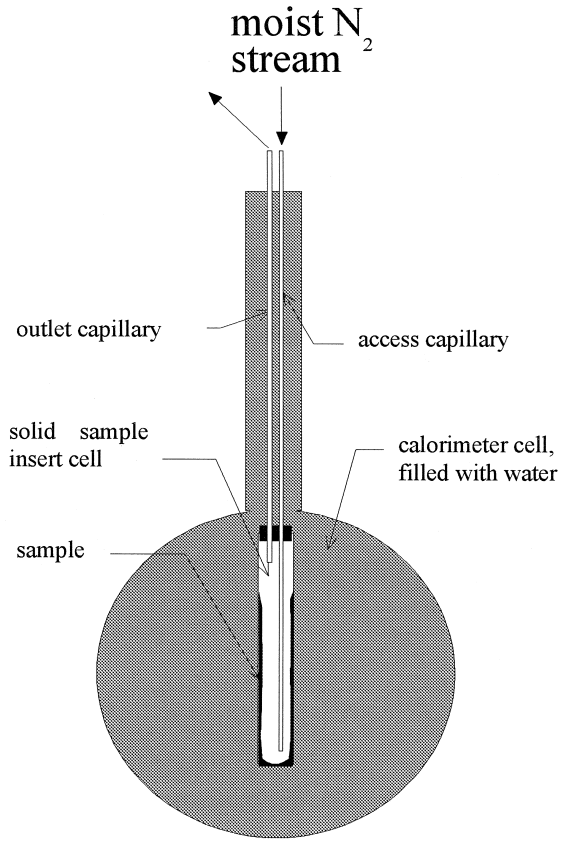

Fig. 1. Schematic representation of the sample compartment of the calorimeter.

Through the cover of the insert cell, two steel capillaries are led out of the calorimeter to allow for the perfusion of the cell content. The access capillary was connected with a moisture generator (HumiVar, Germany) via a thermostated tube. This device evaporates water into a stream of purissimum nitrogen to yield a definite partial pressure of the vapour at isothermal conditions. This way, the moisture generator supplies a permanent N_2 flow of a constant relative humidity (RH) which can be adjusted to any

value in the range 2–98% at 25 ± 0.05°C. The gas passes through the access capillary (~ 25 μl/s) and, additionally, thermally equilibrates before it enters the cell (analogous to the injection needle in the classic liquid-titration arrangement). After perfusing the lipid film, the vapour is led into the room atmosphere through the outlet capillary. The fact that the CFB baseline does not react on variations of the perfusion flux in the range from ~ 50–10 μl/s proves that the perfusion gas is well tempered at the flux conditions used.

Before starting the experiment, the sample was perfused at RH = 2% for 5–10 h for equilibration purposes. Infrared spectra of lipid films which were incubated at identical conditions show no absorption in the spectral range of the O–H stretching band of water (~ 3100–3600 cm^{-1}). We conclude that the lipid is virtually dry because the IR feature is sensitive to the amount of water adsorbed with a resolution of $\sim \pm 0.2$ water molecule per lipid [8,9]. During the calorimetric measurement, the RH is increased in constant steps of, e.g. 2% from 2% to 92%. The moisture generator provides a rate of RH variation of ~ 0.2–0.5%/s in each step. The change in RH initiates the adsorption of water onto the sample. The adsorption heat is compensated by the calorimeter (CFB, cell feedback circuit) to maintain the sample cell at the same temperature as the reference cell which is filled with water. Hence, each step in RH causes a CFB peak due to the adsorption processes, until the system has reached the new equilibrium state. The absolute adsorption heat per RH step, Q, is just the integral over such a peak up from the baseline. Typically, a time of < 15 min was sufficient to ensure equilibration of the sample after a RH step. The whole experiment runs under computer control to give a 'humidity staircase', i.e. the stepwise variation of RH at constant time intervals. A reference measurement using an empty insert cell shows that background effects can be neglected (not shown).

The analysis of the CFB signals (baseline correction, integration) was done by means of self-made software. One should note that the MCS instrument fails to detect slow kinetics of weak amplitude with relaxation times of > 20 min because such effects can hardly be distinguished from baseline instabilities. The fact that the integrated CFB pulses do not significantly depend on the RH increment (ΔRH, see below), the waiting time between subsequent steps and the scan direction (hydration vs. dehydration, not shown) can be considered good evidence for the absence of slow effects up to RH ≈ 90%. A detailed description of the experimental details will be given elsewhere.

2.2. Gravimetry

A lipid film spread on a circular quartz slide (diameter 13 mm) is placed into a twin microbalance system (Sartorius, Germany) which has been equipped with the moisture regulating device described above (see also Ref. [8]). Before starting the experiments, the lipid was dried for 12–24 h at RH = 0% until the mass of the sample attained a constant value (~ 0.5 mg). Adsorption isotherms were recorded in the continuous mode by scanning the RH with a constant rate of $< 10\%$/h throughout the range 0–98% and recording the mass increment.

3. Theory

Every titration experiment is based on the gradual addition of material to a sample in order to push a certain reaction (in the generalised sense) forward. The present method can be viewed as a special titration experiment where each RH step changes the activity of the water, $a_W = \mathrm{RH}/100\%$, in the insert cell by Δa_W. The amount of sorbed water depends on a_W, and thus, each variation of a_W causes the adsorption of

$$\Delta N_W^{g \to s} = N_L \frac{\partial R_{W/L}}{\partial a_W} \Delta a_W \qquad (1)$$

moles of water onto the sample. $R_{W/L}(a_W)$ is the adsorption isotherm

$$R_{W/L} = \frac{N_W^s}{N_L}, \qquad (2)$$

i.e., the number of sorbed (bound) water molecules per lipid (total number N_L).

The calorimetric adsorption experiment can be treated analogously to the ITC binding experiment [10,11], i.e., the transfer of $\Delta N_W^{g \to s}$ water molecules

from the free (gaseous) to the sorbed state gives rise to the heat detected by the calorimeter in each RH step:

$$Q = \Delta N_{\mathrm{W}}^{\mathrm{g}\rightarrow\mathrm{s}} \Delta h_{\mathrm{W}}^{\mathrm{g}\rightarrow\mathrm{s}}, \tag{3}$$

where $\Delta h_{\mathrm{W}}^{\mathrm{g}\rightarrow\mathrm{s}} = h_{\mathrm{W}}^{\mathrm{s}} - h_{\mathrm{W}}^{\mathrm{g}}$ constitutes the difference between the partial molar enthalpy of sorbed water, $h_{\mathrm{W}}^{\mathrm{s}}$, and the molar enthalpy of vapour $h_{\mathrm{W}}^{\mathrm{g}}$ which is considered as a constant reference state.

The normalised, 'observed' heat q_{ITC} is defined by

$$q_{\mathrm{ITC}} \equiv \frac{Q}{N_{\mathrm{L}} \Delta a_{\mathrm{W}}} = \frac{\partial R_{\mathrm{W/L}}}{\partial a_{\mathrm{W}}} \Delta h_{\mathrm{W}}^{\mathrm{g}\rightarrow\mathrm{s}} \tag{4}$$

in order to obtain an experimental quantity which does not depend on the parameters N_{L} and Δa_{W}.

Hence, the partial molar transfer enthalpy of sorbed water $\Delta h_{\mathrm{W}}^{\mathrm{g}\rightarrow\mathrm{s}}$ can be determined without further model assumptions as a function of the degree of hydration of the lipid $R_{\mathrm{W/L}}$, requiring two independent experiments, gravimetry and calorimetry, in order to measure $R_{\mathrm{W/L}}(a_{\mathrm{W}})$ and $q_{\mathrm{ITC}}(a_{\mathrm{W}})$, respectively.

4. Results and discussion

4.1. Gravimetry

Fig. 2 shows the water sorption isotherm of DOPC, $R_{\mathrm{W/L}}(a_{\mathrm{W}})$, and its first derivative, $R'_{\mathrm{W/L}} = \partial R_{\mathrm{W/L}} / \partial a_{\mathrm{W}}$, as obtained from the gravimetric experiment. The lipid swells continuously with increasing RH.

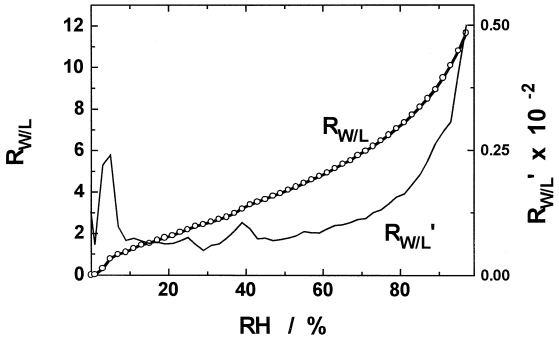

Fig. 2. Adsorption isotherm of DOPC, $R_{\mathrm{W/L}}$, and its slope, $R'_{\mathrm{W/L}}$, at 25°C.

An essentially constant increment of $R_{\mathrm{W/L}}$ in the range $10\% < 80\%$ is followed by a marked increase in the hydration rate at RH > 80%. According to the classification scheme of multimolecular adsorption isotherms [12], lipids with phosphatidylcholine (PC) headgroups exhibit a BET-type [1] IV (or II) character which is indicative for relatively strong water binding [13]. This behaviour is confirmed by the adsorption isotherm of DOPC which closely resembles that of egg yolk lecithin [13]. The slight jump of $R_{\mathrm{W/L}}$ at RH = 40% is caused by a phase transition between a non-lamellar cubic and the lamellar liquid–crystalline phase [14]. Independent infrared linear dichroism measurements on DOPC spread on an attenuated total reflection crystal show a breakdown of the lamellar structure upon dehydrating the lipid at exactly RH = 40% and thus confirm this interpretation of the discontinuity of $R_{\mathrm{W/L}}$(RH) (data not shown, see, e.g., Ref. [15] for details of the method).

4.2. Humidity titration calorimetry

The raw data of the calorimetric hydration experiment are displayed in Fig. 3. Each step upwards in RH causes a negative CFB peak, indicating that the sorption of water from vapour is exothermic. The RH variation inducing the heat response in each step is completed after 10–60 s. Up to RH ≈ 80%, the CFB pulses decay to their baseline value within ~ 2–6 min nearly independently of the RH increment used (ΔRH = 2–20%, cf. Fig. 3). Therefore, we have chosen a time interval of 10–20 min between subsequent RH steps to ensure complete re-equilibration of the system. At RH > 80%, re-equilibration gets slower. However, the time-regime used seems to be appropriate for obtaining reliable data up to RH ≈ 90%. The oscillations of the CFB signal at $6\% < 16\%$ are caused by slight instabilities of the dynamic regime of the moisture generator in the first minutes after reaching the prescribed RH in each step ($\pm 0.5\%$).

An amount of 50 μg of lipid spread uniformly over 1 cm^2 corresponds to a mean stack of 100–150

[1] BET: Brunauer–Emmett–Teller adsorption isotherm.

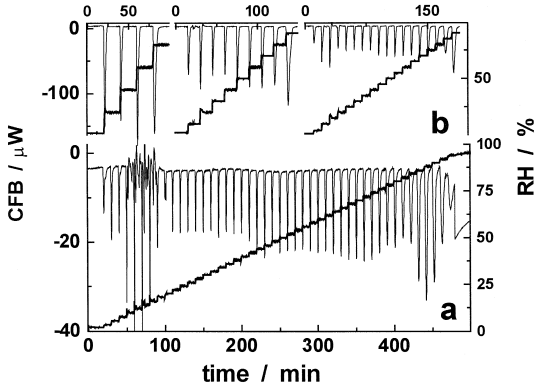

Fig. 3. ITC raw data of the hydration of 50 μg DOPC at 25°C. The increment of RH in each step was 2% (part a), 5%, 10% and 20% (part b, from the right to the left). The RH 'staircase' refers to the right-hand axis and the CFB pulses to the left-hand axis. The time intervals between two subsequent RH steps are 10 min (ΔRH = 2%, 5%), 15 min (10%), and 20 min (20%).

bilayers, i.e. a film thickness of < 1 μm. Huster et al. [16] measured a diffusive water permeability of $P_d = 122$ μm/s for DOPC at 25°C. Jansen and Blume [17] reported that the osmotic water permeability of lipid membranes, which is of relevance for sorption, is more than one order of magnitude faster than the diffusive. Taking these data into account, we may stress that the swelling kinetics are limited by factors other than the water accessibility of the lipids. We suggest that subtle changes of the molecular packing of the lipids are responsible for the relaxation time of the sample film. A detailed study of the hydration kinetics of lipid films is now in progress.

A weak peak of the q_{ITC} curve (ΔRH = 2%) appears just at the RH of the phase transition of DOPC which has been detected by means of gravimetry. It appears reasonable to attribute the heat effect to the same origin. Obviously, the solid support does not affect the phase behaviour of the lipid, and thus, the film possesses the thermodynamic properties of a bulk sample within the resolution limits of the method.

Fig. 4 shows the corresponding differential heats q_{ITC} (cf. Eq. (4)) in comparison with the differential water uptake, $R'_{W/L}$, derived from the sorption isotherm. Both curves run virtually parallel throughout the whole RH range considered. It has been shown previously that the integral heat of water uptake can be used as an experimental parameter to assess $R_{W/L}$ (and $R'_{W/L}$) [18].

Furthermore, the authors derived a BET equation which relates the total heat evolved upon adsorption of a gas on a solid to the activity of the gas. The corresponding model assumes that the sorbed gas molecules assemble into separate layers on a flat surface. Using the designations which have been introduced to describe lipid hydration this expression is given by

$$Q_{int} = \frac{CR^1_{W/L}\left(\Delta h^1_W a_W + \left(\Delta h^\infty_W - \Delta h^1_W\right)a^2_W\right)}{(1-a_W)(1-a_W+Ca_W)}, \quad (5)$$

where $R^1_{W/L} \approx 4$–5 is the 'monolayer coverage', i.e. the number of water molecules which interact directly with the lipid [19], and Δh^1_W and Δh^∞_W are the heats of adsorption of the first and all subsequent adsorption layers, respectively. The constant $C = \exp\{-(\Delta h^1_W - \Delta h^\infty_W)/RT\}$ is the same as in the classical BET equation. Differentiation of Eq. (5) with respect to a_W and extrapolation to $a_W = 0$ gives

$$q_{ITC}(a_W \to 0) \equiv \frac{\partial Q_{int}(0)}{\partial a_W} = CR^1_{W/L}\Delta h^1_W. \quad (6)$$

Eq. (6) yields the solution $\Delta h^1_W - \Delta h^\infty_W \approx 12 \pm 4$ kJ/mol for the range 10% < 60% based on the experimental value $q_{ITC}(a_W \to 0) \approx 0.75 \pm 0.2$

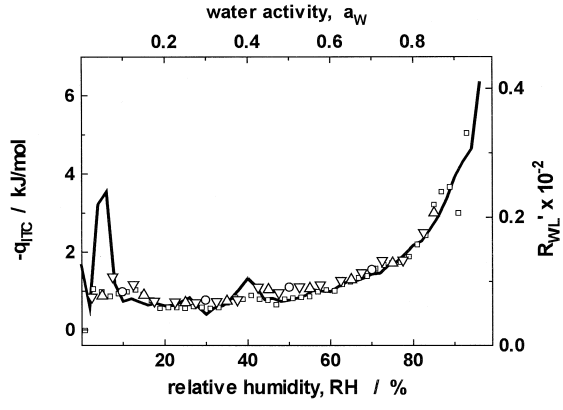

Fig. 4. Observed adsorption heat per pulse, q_{ITC} (open symbols), and rate of water adsorption, $R'_{W/L}$ (line), of DOPC as a function of the relative humidity. q_{ITC} is obtained by integrating the CFB pulses corresponding to ΔRH = 2% (□), 5% (▽), 10% (△) and 20% (○) (cf. Fig. 3).

kJ/mol and $h_W^\infty \approx \Delta h_W^{g\to b} = -44.6$ kJ/mol, the condensation heat of bulk water.

4.3. Combining gravimetry and calorimetry

The difference $(\Delta h_W^1 - \Delta h_W^\infty)$ represents a measure of the mean partial molar enthalpy of the water which binds directly to the lipid according to the model assumptions. The combination of gravimetric and calorimetric experiments offers, however, the possibility to determine this quantity by means of Eq. (4) in a model independent way. Fig. 5 depicts the difference

$$\Delta h_W^{b\to s} = \Delta h_W^{g\to s} - \Delta h_W^{g\to b} \quad (7)$$

as a function of $R_{W/L}$, the number of water molecules adsorbed per lipid. The choice of bulk water as the reference state is motivated by the fact that biomembranes exist usually under excess water conditions. Hence, $\Delta h_W^{b\to s}$, gives the partial molar enthalpy of water upon transfer from the aqueous bulk phase into the hydration shell of the lipid. It is endothermic at $R_{W/L} < 6$, i.e. in the range which corresponds to the hydration layer next to the lipid headgroups [19]. The mean value over this range of 10–15 kJ/mol agrees with the rough estimate of $\Delta h_W^1 - \Delta h_W^\infty$ given above. Enthalpically, water molecules sorbed beyond $R_{W/L} = 6$, i.e. in the range of the second hydration layer, behave like bulk water within the error limits. The distinct decrease of $\Delta h_W^{b\to s}$ at $R_{W/L} = 1$–2 reveals, probably, the existence of strong primary binding sites of water such as the phosphate groups. Note, however, that the experimental uncertainty increases considerably at $R_{W/L} < 2$. Consequently, this interpretation is speculative at present.

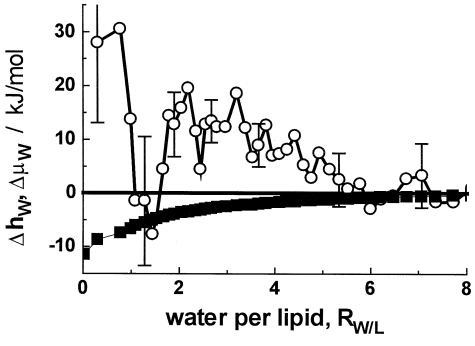

Fig. 5. Partial molar enthalphy of hydration, $\Delta h_W^{b\to s}$ (○, cf. Eq. (7)) and chemical potential of hydration, $\Delta \mu_W^{b\to s}$ (■, cf. Eq. (8)), of DOPC as a function of the number of water molecules sorbed per lipid. ($T = 25$°C).

4.4. Thermodynamics of water adsorption onto DOPC

The chemical potentials of the coexisting gaseous and sorbed water are equal in the experiment due to thermodynamic equilibrium, i.e. $\Delta \mu_W^{g\to s} = \mu_W^s - \mu_W^g(a_W) = 0$ (and $\Delta \mu_W^{g\to b} = \mu_W^b - \mu_W^g(1) = 0$). It follows immediately that

$$T\Delta s_W^{g\to s} = \Delta h_W^{g\to s} \quad \text{and} \quad T\Delta s_W^{g\to b} = \Delta h_W^{g\to b},$$

where $\Delta s_W^{g\to s}$ and $\Delta s_W^{g\to b}$ are the entropy changes of water upon adsorption ($a_W < 1$) and condensation ($a_W = 1$), respectively. The two equations can be combined to give

$$T\left(\Delta s_W^{g\to s} - \Delta s_W^{g\to b}\right) = \Delta h_W^{g\to s} - \Delta h_W^{g\to b}.$$

Rearrangement yields

$$\Delta \mu_W^{b\to s} = \Delta h_W^{b\to s} - T\Delta s_W^{b\to s} = \mu_W^g(a_W) - \mu_W^g(1) = RT \ln a_W. \quad (8)$$

This expression relates the water activity which is adjusted in the adsorption experiment to the changes of enthalpy, entropy and chemical potential upon transferring water from the bulk phase into the hydration shell of the lipid.

Fig. 5 depicts $\Delta \mu_W^{b\to s}$ as a function of $R_{W/L}$. Its negative values reflects the property of lipids with PC headgroups to hydrate spontaneously. Eq. (8) shows that the endothermic transfer enthalpy ($\Delta h_W^{b\to s} > 0$) at $R_{W/L} < 6$ is paralleled by a positive entropy term $T\Delta s_W^{b\to s} > 0$.

That means that according to the enthalpy no spontaneous hydration of the lipid could occur from bulk water because the enthalpy gained by the lipid–water interactions is lower than that lost upon disturbing or breaking water–water and/or lipid–lipid interactions. The fact that the lipid does spontaneously swell shows that it is the entropy which drives the hydration by overcompensation of the enthalpic penalty. Hydration of DOPC at room temperature is accompanied by an increase of the motional freedom of the bound water, of the polar and of the apolar moieties of the lipid upon water adsorption [20,21]. Progressive hydration leads usually to

an increased exposure of the hydrophobic region of the lipid aggregates to the water. This tendency is thermodynamically unfavourable due to the hydrophobic effect. Consequently, it counteracts the spontaneous swelling of the lipid and finally gives rise to a maximum size of the hydration shell of the lipid.

5. Conclusions

The humidity titration technique enables the study of the thermodynamics of hydration with molecular resolution. That is, the enthalpy, entropy and free energy of water binding to distinct hydration sites can be determined experimentally.

The hydration of DOPC at room temperature is entropy driven. That means the disordering effects enabled by the water dominate over ordering effects.

Because of its high sensitivity, the method is expected to give considerable insight into the mechanisms driving the hydration of biologic materials such as lipids, proteins and nucleic acids. The time resolution of the order of seconds gives the opportunity to investigate kinetic aspects of hydration in detail. Time-dependent hydration phenomena are, for example, important issues in food and drug research.

Acknowledgements

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