The role of phospholipid headgroup composition and trehalose in the desiccation tolerance of *Caenorhabditis elegans*

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Supporting Information

Organic extraction, separation and analysis of *C. elegans* lipids

Native *C. elegans* phospholipids were extracted from preconditioned and non-preconditioned dauer larvae. Frozen worm suspensions were thawed in an ultrasound water bath for 5 min and frozen again in liquid nitrogen, in total five times for efficient homogenization. Total soluble protein amounts were measured by BCA assay (Thermo Scientific, USA) at this stage when necessary. Subsequently, homogenates were extracted via Bligh and Dyer's method ¹. Briefly, 1 ml of homogenate in water was mixed with 3.75 ml of CHCl₃:CH₃OH (1:2, v/v) for at least 25 min and then centrifuged at 3000 g for 5 min to pellet cuticles and non-soluble material. The supernatant was transferred into a fresh tube and added 1.25 ml CHCl₃. To induce phase separation, 1.25 ml H₂O was finally added. After thorough mixing, phase separation was accelerated by centrifugation at 3000 g for 10 min. This yields an upper aqueous phase, a lower lipid-enriched organic phase and a thin interphase that contains mostly proteins and DNA. The lower phase is transferred to a tube, dried under N₂ flow and dissolved in CHCl₃:CH₃OH (2:1, v/v).

The total organic phase of extracts were separated into three fractions via solid phase extraction (SPE) by loading it on a CHCl₃-equilibrated silica gel column and eluting subsequently with CHCl₃, (CH₃)₂CO:CH₃OH (9:1, v/v) and CH₃OH. Dry weights of each fraction were measured for normalization. Separation was verified by thin layer chromatography on 10 cm HPTLC plates (Merck, Darmstadt, Germany) using CHCl₃:CH₃OH:H₂O (45:18:3, v:v:v) as the mobile phase. Only the CH₃OH fraction, which is enriched in phospholipids and relatively hydrophilic lipid species, was used in subsequent experiments. Radioactively labeled lipid fractions were normalized by scintillation counting

whereas non-labeled lipid fractions were normalized by dry weight or total soluble protein amount in the original homogenate.

For qualitative chemical analysis of phospholipids, equivalent amounts of total lipid extracts or methanol fractions of SPE were separated on 2 dimensional thin layer chromatography (1st dimension $\text{CHCl}_3:\text{CH}_3\text{OH}:\text{NH}_3(32\%)$ (65:35:5, v/v/v); 2nd dimension $\text{CHCl}_3:\text{CH}_3\text{OH}:(\text{CH}_3)_2\text{CO}:\text{CH}_3\text{COOH}:\text{H}_2\text{O}$ (50:10:20:12.5:5, v/v/v/v/v)). Radioactively labeled lipids were visualized by exposing the TLC plate on an X-ray film for 2 weeks. Other TLC plates were sprayed with 3% (w/v) $\text{Cu}(\text{CH}_3\text{COOH})_2$ in 8% (v/v) H_3PO_4 and baked at 180 °C to visualize all lipid species with at least one double bond in the hydrocarbon chain.

Vesicle preparation

The organic solvent was removed from lipid extracts by drying under nitrogen gas followed by vacuum-drying for at least 2 h and re-suspension in water (1 mg/ml). Vesicles were mixed rigorously every 10 minutes during 2 h incubation at 60 °C followed by 15 cycles of liquid nitrogen freezing and thawing. Uniform vesicles were prepared by extrusion of the suspension through a 100-nm polycarbonate filter using a LiposoFast extruder (Avestin, Ontario, Canada). 20 μL of the vesicles suspension (~3 mM lipid) was either supplemented with 2 μL of a 0.15 mM trehalose solution or spread directly on the diamond surface of an attenuated total reflectance ATR-crystal (RESULTEC, Illerkirchberg) and dried under nitrogen gas.

Spectral Processing

a) Correction for swelling of PL films upon transient hydration. The time-resolved absorption spectra monitor the transient water uptake and release via the absorption increase

of the water OH stretching $\nu(\text{OH})$ and scissoring bands. They are additionally affected by a general decrease of lipid absorption due to the swelling of the lipidic film upon water uptake in ATR geometry². All spectra were corrected for swelling as described previously³. Briefly, the reference absorption spectrum of the lipidic film was re-scaled for each time-slice to minimize the integral absorption change between 1360 and 800 cm^{-1} . This is equivalent with conservation of total absorption in this spectral range which shows minimal with water absorption. The corresponding weighing factors scale linearly with the volume of the sample at each time-slice in accordance with the use of thin absorbing films². The hydration-induced water OH scissoring band was subtracted from the difference spectra (using a spectrum of liquid water) to better resolve underlying $\nu(\text{C}=\text{O})$ bands. Likewise, the low frequency part of the water $\nu(\text{OH})$ was subtracted to reveal the changes of the symmetric ($\nu_{\text{s}}(\text{CH}_2)$) and antisymmetric ($\nu_{\text{as}}(\text{CH}_2)$) methylene stretching modes of the acyl chains. All spectral correction procedures were performed automatically using an Origin 9 script. The relative water content of the samples was determined from the intensity of the water $\nu(\text{OH})$ band relative to the asymmetric PO_2^- absorption as described before⁴ and expressed as hydration Γ in $(\text{mol H}_2\text{O})/(\text{mol lipid})$.

Figure S1 shows the high reproducibility of the sample responses to ten successive hydration pulses, showing that the variations of the absorption changes are within the spectral noise. The latter is ± 0.3 mAU for the $\nu(\text{CH}_2)$ in the raw data and it is smaller in all other evaluated spectral regions. After averaging over ten experiments, the noise is further reduced by a factor of ~ 3 . This results in a final uncertainty of the spectral amplitudes of less than 6 %.

The data also evidence the return to equilibrium before application of another hydration pulse. In fact, this fully reversible spectral behavior shown in Fig. S1 was the criterion for the adjustment of the waiting time (8 min) between hydration pulses.

Figure S1: Raw data of hydration-induced difference spectra. The plot shows the overlay of ten difference spectra, each induced in NPLs by an independent hydration pulse (water background subtracted from 3000 to 800 cm^{-1}). Each difference spectrum has been recorded 19 s after the hydration pulse, corresponding to the midpoint of the time traces shown in Figs. 2 and 5 in the main text. The spectra are reproduced within the noise level, evidencing the high degree of reproducibility and the full relaxation of the sample after each hydration pulse.

b) Determination of hydration-induced volume changes of PL films. The relation between total absorption and film thickness in ATR geometry² allows deriving the relative expansion $\Delta V/V$ of the PL films at each of the time-resolved hydration levels. In fact, these values are the above used scaling factors of the reference spectrum used to correct for the swelling-induced absorption decrease. The slope of their plot versus hydration Γ ((mol H_2O)/(mol lipid)) represents the relative molar expansion coefficient $\Delta V \cdot V^{-1} \cdot \Delta \Gamma^{-1}$ per uptake of one water molecule per lipid as shown in Fig. S2 (A and B). This coefficient is nearly identical for NPLs and PPLs in the absence of trehalose (Fig. S2A), whereas in the presence of trehalose,

Figure S2: Time-resolved volume and H-bond changes. Relative expansion of PL films in the absence (A) and presence (B) of trehalose. The evaluation is based on the time-dependent data in Figs. 2 and 5, respectively. The sequence of swelling / shrinking is indicated by the selected time points, the slopes α are relative expansion coefficients ($\alpha = \Delta (\Delta V/V) / \Delta \Gamma$, standard error $< 10^{-2} \%$). C) $\nu(\text{OH})$ contour of water adsorbed during the hydration pulse in the absence (blue) and presence (red) of trehalose. D) Hydration-induced absorption changes of trehalose in PLs. Color code: black, NPLs; green, PPLs.

the swelling of PPLs is significantly larger than in NPLs (Fig. S2B). The determined relative expansion coefficients are related to the material properties of the PL film as a whole, i.e., they also contain the volume effects of inter bilayer water. This 3D expansion analysis differs from the analysis of purely lateral expansion by which bilayers respond to hydration. The latter is determined

by the compressibility module for which independent estimates have been obtained as detailed below. Figure S2C shows that the transiently absorbed water forms an additional population with higher H-bond strength when trehalose is present in the PL films. This gives rise to the low frequency shoulder in the $\nu(\text{OH})$ contour. The direct participation of trehalose in remodeling of the H-bond network is evident from its hydration-induced difference bands (Fig. S2D).

c) Estimation of the compressibility modulus of PL films. Using the linear relation between the frequency shift of the symmetric methylene stretching mode and the area per lipid ⁵: $\Delta\nu_s(\text{CH}_2) = (9.7 \text{ cm}^{-1} / \text{nm}^2) \cdot \Delta A_L$, the spectral data were evaluated to get an estimate of the compressibility modulus K_c according to the relation ⁶:

$$\Delta A_L = 2 \cdot RT \cdot \Delta(\Gamma \cdot \ln(a_w)) / K_c. \quad (\text{Eq 1})$$

The time-dependent activity of water at each of the time-points at which difference spectra were recorded was linearly approximated by:

$$a_{wi} = 0.75 + \Delta RH \cdot \Delta\Gamma(t_i) / \Delta\Gamma(t_1), \quad (\text{Eq 2})$$

where ΔRH is the initially induced increase of RH after the hydration pulse (here 7%) at time t_1 and $\Delta\Gamma(t_i)$ is the number of excess water molecules per lipid at time t_i relative to the initial hydration $\Gamma(t_0)$. From the amplitude $\Delta A(\text{CH}_2)$ of the $\nu_s(\text{CH}_2)$ difference band, the underlying frequency shift was derived by the relation $\Delta\nu_s(\text{CH}_2) = 0.35 \times \Delta A(\text{CH}_2) / A(\text{CH}_2)$, where $A(\text{CH}_2)$ is the total absorption of the $\nu_s(\text{CH}_2)$ in the reference spectrum before the hydration pulse. This relation is easily derived for band shifts that are small as compared to the full width at half maximum (FWHM) of a gaussian band. The experimentally determined FWHM of the $\nu_s(\text{CH}_2)$ at 2850 cm^{-1} is 20 cm^{-1} .

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