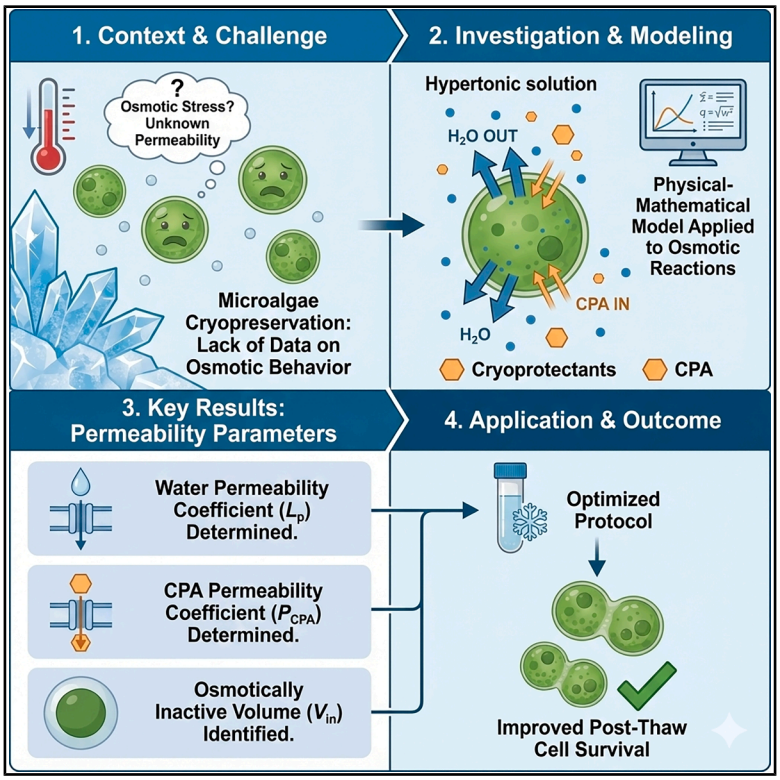
iScience

Article

Permeability of the plasma membrane to water and cryoprotectants of *Coelastrella thermophila var. globulina* microalgae cells

Graphical abstract



Highlights

- Osmotic behavior of C. thermophila var. globulina during freezing and thawing was analyzed
- Osmotically inactive cell volume determined for this microalgae species
- Permeability coefficients for water and cryoprotectants calculated via a model
- Findings help optimize cryopreservation protocols for better post-thaw survival

Authors

Nadiia Chernobai, Nadiia Shevchenko, Igor Kovalenko, Leonid Rozanov

Correspondence

nadiiachernobai@gmail.com

In brief

Membrane architecture; Microbiology; Cell biology

Chernobai et al., 2026, iScience *29*, 114594
February 20, 2026 © 2026 The Authors. Published by Elsevier Inc.
https://doi.org/10.1016/j.isci.2025.114594

CellPress

iScience

CellPress
OPEN ACCESS

Article

Permeability of the plasma membrane to water and cryoprotectants of *Coelastrella thermophila var. globulina* microalgae cells

Nadiia Chernobai,[1,2,3,*] Nadiia Shevchenko,[1] Igor Kovalenko,[1] and Leonid Rozanov[1]
[1]Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, 61016 Kharkiv, Ukraine
[2]National Scientific Center "Institute of Forensic Expertise named after Ex. Prof. M. S. Bokarius" of the Ministry of Justice of Ukraine, 61177 Kharkiv, Ukraine
[3]Lead contact
*Correspondence: nadiiachernobai@gmail.com
https://doi.org/10.1016/j.isci.2025.114594

SUMMARY

The study of osmotic reactions of cells during freezing and thawing is a crucial topic requiring detailed investigation. Understanding these processes allows for the optimization of cryopreservation protocols, thereby improving cell survival after thawing. While the literature provides information on the osmotic characteristics of various mammalian cells, data on plant cells, including microalgae, remain scarce. This article examines the osmotic behavior of microalgae cells, which are promising from a biotechnological perspective, during freezing and thawing. We obtained results on the osmotically inactive cell volume and, using a physical-mathematical model, determined the permeability coefficients of cells for water and cryoprotectants. These results will be used in further work on developing cryopreservation protocols for microalgae, enabling the optimization of the process and achieving the best possible post-thaw survival outcomes.

INTRODUCTION

Cryopreservation is used for the storage of cells, tissues, and organs. It is applicable for long-term storage of algae, including most cyanobacteria and eukaryotic soil microalgae, many freshwater and marine eukaryotic chlorophyte algae, marine diatoms, chlorarachniophytes, eustigmatophytes, pelagophytes, phaeothamniophytes, and ulvophytes.[1] They exhibit vastly different physical responses to cooling/warming and, thus, require unique individual cryopreservation protocols for optimal storage. For many microalgae, effective cryopreservation methods have yet to be developed; however, some generalizations on how to cryopreserve a particular species of algae can be made based on structural, biochemical, and biophysical features of the species.

Computational biophysical modeling may help to determine very important biophysical characteristics of cells, such as the osmotically inactive fraction, hydraulic conductivity, cryoprotectant (CPA) penetration coefficients, activation energies for water and CPA transport across the plasma membrane.[2,3] The models are based on the assumptions that the cell membranes are spherical and that the membrane's physical characteristic does not shift cooperatively on changing temperature. The models of the transport of solutes across the plasma membrane were tested on many mammalian cells.[4–6] They were used to determine the optimal duration of incubation of cells with CPAs, incubation temperature, methods of CPA addition/removal, and cooling/warming rate. This, in turn, minimized cell damage and maximized cell viability after cryopreservation. The data for algae species are scarce due to their specific structural features, for example, the presence of the cell wall, specific membrane proteins, and selective channels.[7] It is a question of how the above-mentioned models would predict the osmotic behavior of algae and whether they could come in handy for the cryopreservation of some algae species. Cell permeability to water and CPAs plays a key role in cryopreservation. It predetermines the rate of the saturation of cells with permeating CPAs or of dehydration by impermeating ones, or by growing concentration of solutes during extracellular water crystallization.[8–10] Moreover, the effectiveness of using CPAs depends on the correct choice of its concentration, exposure time, and temperature, as well as an understanding of the mechanisms of their penetration through the cell membrane.

Coelastrella thermophila var. *globulina* (Scenedesmaceae, Chlorophyta) is a thermophilic green microalga isolated from a hot spring with a temperature of about 63 °C. It is capable of changing cell color from dark green to brick-red or orange during growth, associated with carotenoid accumulation. The species has a balanced fatty acid composition, including substantial amounts of oleic, palmitic, linoleic, and alpha-linolenic acids, as well as rare gamma-linolenic and stearidonic acids, which are precursors of arachidonic and eicosapentaenoic acids. Due to its high content of biologically valuable polyunsaturated fatty acids and tolerance to extreme conditions, this strain is considered a promising source of food and feed additives, as well as a raw material for biodiesel production with favorable performance characteristics.[11,12]

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

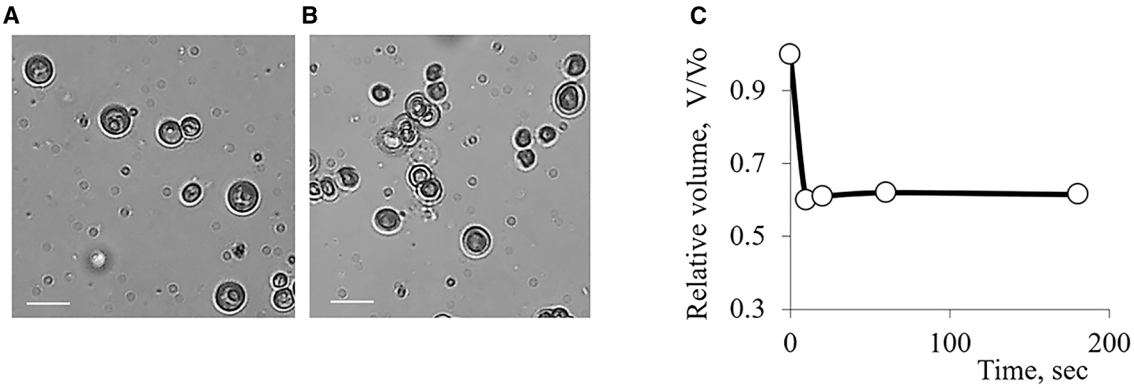


Figure 1. Osmotic behavior of cells

(A–C) Cells in 0.5 M NaCl (isotonic) (A) and in 1.7 M NaCl (hypertonic) (B) at 25°C after 60 s; time-dependent cell volume contraction (C).

Cryopreservation of *Coelastrella thermophila* var. *globulina* (*C. thermophila* var. *globulina*) would ensure the availability of its strains for reproduction at any time, regardless of seasonal or climatic conditions, and allows the preservation of *C. thermophila* var. *globulina* strains with specific properties for continuous use. Due to its spherical shape, the microalgae *C. thermophila* var. *globulina* may be a convenient model organism for studying the osmotic behavior of cells at different stages of freezing and thawing.

The objective of the research is to determine the transport characteristics of the *C. thermophila* var. *globulina* plasma membrane (permeability coefficients for water and CPA molecules) using the Kedem-Katchalsky model. The work can potentially clarify the applicability of the Kedem-Katchalsky model of membrane transport for *C. thermophila* var. *globulina* and to contribute to the creation of new ways of long-term preservation of algae species.

RESULTS

Osmotically inactive fraction measurement

Osmotically inactive fraction of *C. thermophila* var. *globulina* cells are one of the most important characteristics of the cell that determines its osmotic behavior, as it is one of the phenomenological coefficients of the modified Kedem-Katchalsky model.[2,3]

Placing *C. thermophila* var. *globulina* cells in a hypertonic NaCl solution (3.4 M) led to membrane shrinkage after 10 s incubations (Figure 1). The minimal volume remained constant, which points to the impermeability of the membrane for Na^+ and Cl^- ions.

The *C. thermophila* var. *globulina* cells dehydrated in solutions with hypertonic concentrations (1–3.4 M). The cell volume exhibits a linear relationship with the inverse of the osmolyte concentration in the solution, consistent with the Boyle-Van't Hoff relation.[13] (Figure 2). Extrapolation of cell volume to the *y* intercept of the graph shows that the nonosmotic fraction of *C. thermophila* var. *globulina* cells are 0.6 ± 0.002 or 60 ± 0.2% of their isosmotic volume.

Membrane transport characteristics of *C. thermophila* var. *globulina*

Methanol and Me_2SO have been commonly used as CPAs for the cryopreservation of algae.[14] However, both methanol and Me_2SO can be toxic to algae at high concentrations.[15] Therefore, in this work, other widely used CPAs for plant cells were examined glycerol, EG, 1,2-propanediol, ethanol, and sucrose. These CPAs are considered permeable through the cell wall and the membrane except sucrose, which can cross the cell wall easily but not the plasma membrane, and requires special transporters to be transferred.[16–18] Our studies have shown that the cells, at first, gradually dehydrated in sucrose solutions during 100 s at 25°C losing about a third of their initial volume. However, further exposure resulted in the partial recovery of their initial fraction up to 0.9. This indicated penetration of sucrose molecules through the cell membrane of the microalgae *C. thermophila* var. *globulina* (Figures 3A and 3B).

When CPAs are introduced to a culture of *C. thermophila* var. *globulina*, water moves out faster than the CPAs move in, resulting in a transient shrinkage of the cells (Figures 4A–4E). Maximum dehydration of cells was observed within the range of 10–30 s, except for ethanol, for which it was completed within 10 s.

In some cells (about 10%), besides the compression of the plasma membrane, plasmolysis and deplasmolysis were observed (Figure 5). The extent of plasmolysis reached 30% of the initial cell fraction. Deplasmolysis was observed at 60–90 s of exposure, depending on the investigated CPA solution.

Rehydration in ethanol, EG, and Me_2SO solutions was completed in 60 s, at 1.2 PD in 100 s. The volume recovery time in glycerol solution was comparable to that in sucrose and was completed in 300–400 s.

Based on the osmotic behavior, the kinetics of the volume changes in *C. thermophila* var. *globulina* cells and theoretical curves extrapolating the osmotic behavior of the cells (Figures 6A–6E), Lp and K_1 for various CPAs were obtained (Table 1).

DISCUSSION

Cryopreservation of microalgae is crucial for biodiversity conservation, rapid reproduction of bioresources, and biotechnology. So far, the majority of microalgae species and strains have not been successfully cryopreserved, and there are no reports of successful cryopreservation of *C. thermophila* var. *globulina*. We believe this is due to a lack of knowledge regarding the

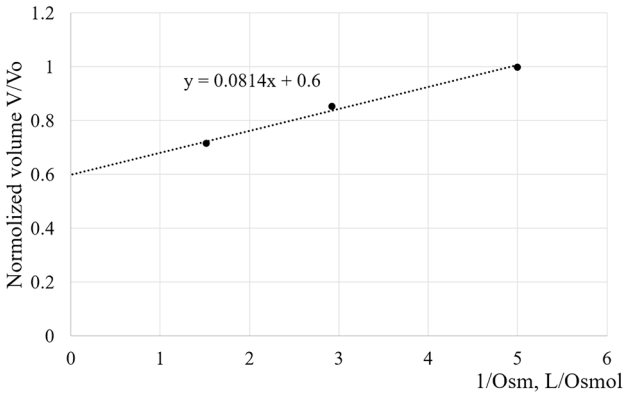


Figure 2. Boyle-Van't Hoff relationships for *C. thermophila var. globulina* cells at 25°C

Extrapolation of cell volume to the *y* intercept of the graph shows that the nonosmotic fraction of *C. thermophila var. globulina* cells is 60 ± 0.2% of their isosmotic volume.

cryobiological characteristics of such specimens. According to Mazur's theory, freezing of living cells involves the transfer of heat and mass. Studying the individual biophysical parameters of each cell type allows us to find the optimal cryopreservation protocol. Therefore, optimizing the cryopreservation of *C. thermophila* var. *globulina* cells require understanding sample properties such as the osmotically inactive cellular fraction and membrane permeability to water and CPAs.

Osmotically inactive fraction of *C. thermophila* var. *globulina* cell determines the proportion of intracellular substances that are unable to leave the cell without damaging the membrane, including biological macromolecules (nucleic acids, proteins, and peptides), membrane structures, cytoskeletal structural elements, bound water, and others. It is possible that *C. thermophila* var. *globulina* cells with a sufficiently high osmotically inactive fraction will dehydrate faster during exposure to CPAs and on freezing than cells with a lower osmotically inactive fraction. This suggests shorter incubation times with CPAs and the use of rapid cooling rates.

Plant cells, including *C. thermophila* var. *globulina*, are surrounded by the cell wall that restricts the free passage of water and dissolved substances. This significantly distinguishes them from mammalian cells, where there are no limitations on the passage of solvent through the membrane. Additionally, plant cells experience turgor pressure,[1,19] which arises due to the accumulation of water in the vacuoles. This pressure can vary depending on osmotic pressure and the activity of vacuolar membranes.

Another possible distinctive feature of *C. thermophila* var. *globulina* cells, which may distinguish them from the conventional model, is the potential permeability of their membrane to sucrose molecules. Given that sucrose is a large polar solute, its ability to cross the hydrophobic core of the membrane would typically require the involvement of specific membrane proteins— sucrose transporters (SUTs). Such transporters are known to be present in some plant cells, including certain soil algae.[20] The observed phenomena may therefore indicate, though not yet confirm, the presence of a similar transport system in *C. thermophila* var. *globulina*.

The apparent ability of *C. thermophila* var. *globulina* cells to undergo plasmolysis and deplasmolysis could represent a protective mechanism that helps minimize structural damage during dehydration. This process might contribute to maintaining cell functionality, regulating osmotic pressure, and promoting survival under stress conditions.

The values for Lp obtained in this study (1.16–7.22)×10^{-14} $m^3s^{-1}N^{-1}$ at 25°C, fall within the broad range of permeability values measured in other algal cells. Data from this study are close to the value of Lp (1.85 ± 0.27)×10^{-14} $Nm^{-3}s^{-1}$ found for *Chlorococcum texanum* and *Valonia ventricose*.[21,22] Values of Lp recorded in other algae span two orders of magnitude, from 10^{-14} $m^3s^{-1}N^{-1}$ to 10^{-12} $m^3s^{-1}N^{-1}$.

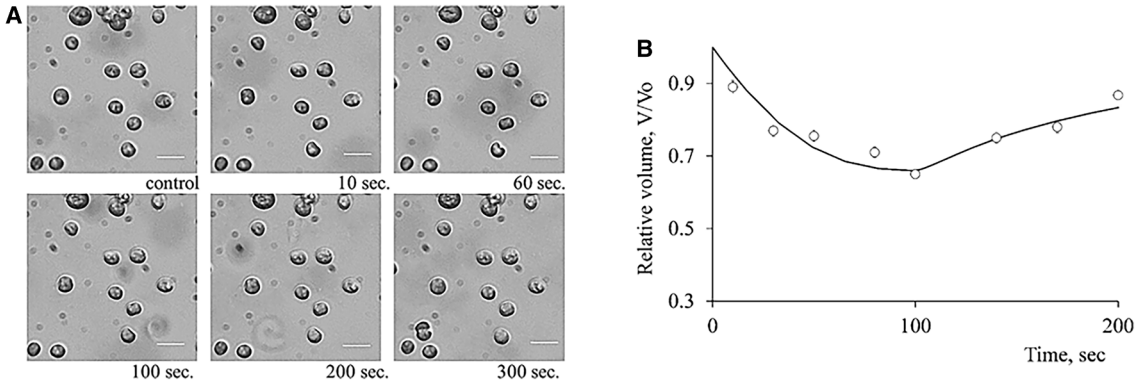


Figure 3. Osmotic behavior, kinetic changes, and volume responses of C. thermophila var. globulina cells under different experimental conditions

(A) Osmotic behavior and kinetic changes in the volume of *C. thermophila var. globulina* cells in response to the addition of 1 M sucrose at 25°C (scale bars = 20 μm).

(B) Experimental data (marker) and theoretical dependencies (curve) of the volume change of spherical *C. thermophila var. globulina* cells vs. time in 1 M sucrose solution at 25°C.

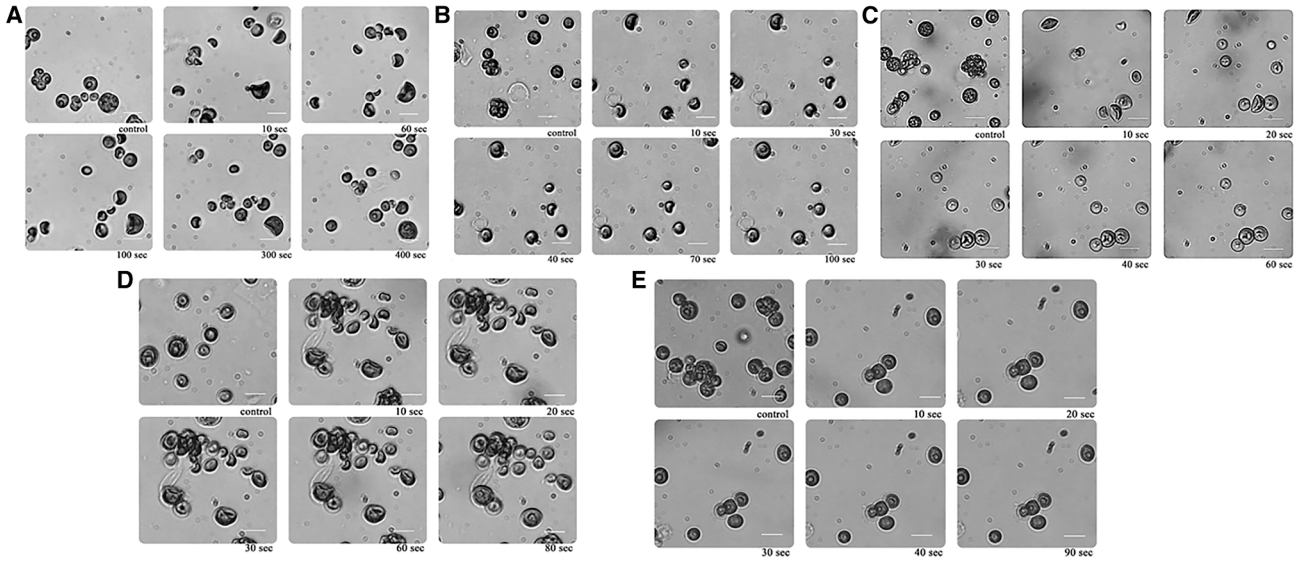


Figure 4. Osmotic behavior and kinetic changes in the volume of *C. thermophila var. globulina* cells at 25°C in response to the addition of cryoprotectant solutions (scale bars = 20 μm)

(A) 3.4 M glycerol solution.
(B) 3.4 M 1.2-PD solution.
(C) 3.4 M Me_2SO solution.
(D) 3.4 M EG solution.
(E) 3.4 M ethanol solution.

The K_1 obtained for *C. thermophila* var. *globulina*, range from 10^{-8} to 10^{-5} $m^3s^{-1}N^{-1}$, which is consistent with the findings of other authors.[6,23]

The modeling predicts that the plasma membrane of *C. thermophila* var. *globulina* is more permeable to ethanol molecules compared to other studied substances. Ethanol has a relatively small size and molecular weight, and the presence of only one hydroxyl group (-OH) allows it to pass through the lipid bilayer more easily compared to larger or highly polar molecules, which facilitates its diffusion through membranes. Glycerol and sucrose molecules had several orders lower penetration coefficients compared to other CPAs. Despite the fact that the glycerol molecule has a relatively small size, its polar groups may create barriers for simple diffusion across the hydrophobic lipid layer of the membrane. Despite the small size of the EG molecule, it may penetrate the plasma membrane less efficiently due to the presence of two hydroxyl groups and the absence of specific transport proteins. The permeability for Me_2SO was comparable to that for EG. Despite the polarity of the molecule, it has a relatively small size, as well as a sulfoxide group that provides high solubility in water and polar environments and may facilitate its interaction with cell membranes and ease its penetration.

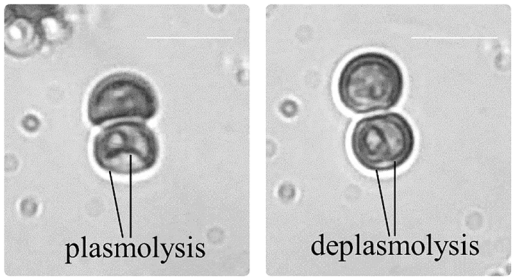


Figure 5. Plasmolysis and deplasmolysis of *C. thermophila var. globulina* cells in response to the addition of 3.4 M ethanol solution at 25°C (scale bars = 20 μm)

Plasmolysis and deplasmolysis of *C. thermophila var. globulina* cells in response to the addition of 3.4 M ethanol solution at 25°C (scale bars = 20 μm).

It has been previously shown that the incubation of unicellular freshwater green microalgae *Chlorococcum dissectum* with ethanol and Me_2SO solutions for 60 min reduced the metabolic activity of the cells (culture) by 31–33%, the ethylene glycol ones did by 50%. The least damaging effect was obtained in 10% glycerol solution.[10] Based on the obtained data on the permeability of *C. thermophila* var. *globulina* cells, it is possible to recommend a short-term incubation from 1 to 7 min (depending on the CPA) with the studied CPAs before cryopreservation. This will allow the CPAs to achieve a protective effect, significantly reducing the toxic effect on cells during freezing/thawing.

Thus, it can be stated that the microalgae C. thermophila var. globulina have a highly permeable cell membrane to water and CPAs, allowing the cells to adapt quickly to changes in osmotic pressure, thereby minimizing damage during the freezing/thawing process with the use of CPAs.

The obtained data will allow us to optimize CPA protocols for *C. thermophila* var. *globulina* cells by determining the optimal incubation time with CPAs and rates of cooling/warming.

Future work should address unresolved aspects of transmembrane transport of small molecules, including ethanol and ethylene glycol, as well as the potential role of sugar transporters

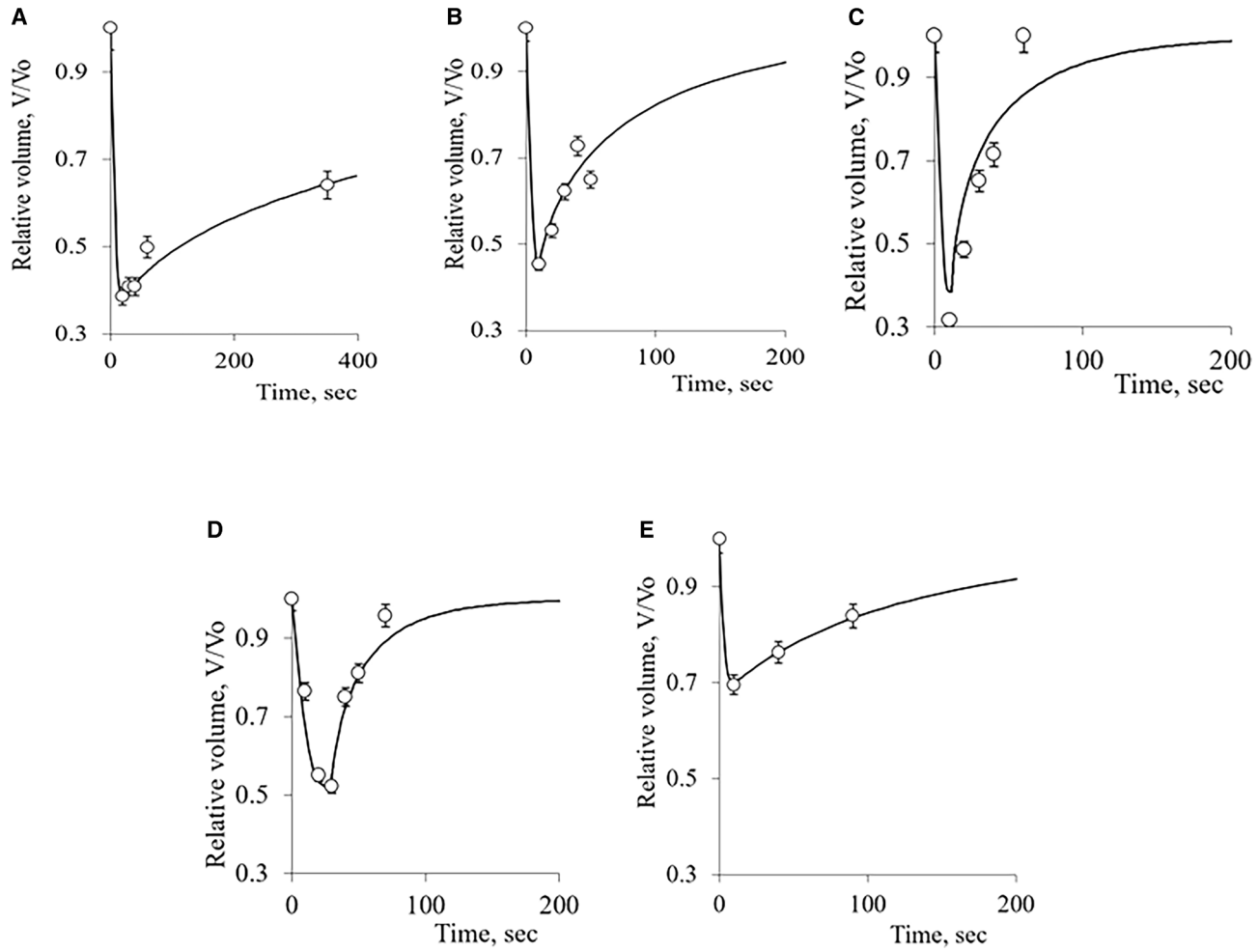


Figure 6. Volume change of *C. thermophila var. globulina* cells vs. time in response to the addition of cryoprotectant solutions

Experimental data (dots) and theoretical dependencies (curves) of volume change of *C. thermophila var. globulina* cells vs. time in 3.4 M glycerol (A), 3.4 M 1.2-PD (B), 3.4 M Me_2SO (C), 3.4 M EG (D), and 3.4 M ethanol (E) solutions.

in microalgal cells. Integrating genomic, biophysical, and cryomicroscopic approaches will be crucial for a deeper understanding of cellular responses during freezing and thawing, for the optimization of cryopreservation protocols, and for investigating the temperature dependence of transmembrane transport. Also, in further work, additional experiments are planned to assess cell viability and metabolic activity after thawing, which will help increase the reliability of the recommendations concerning the incubation time of cells with CPAs prior to freezing.

Table 1. Permeability coefficients of *C. thermophila var. globulina* cells for water and CPAs at 25°C

CPAs	Molecular diameter, nm	$Lp \times 10^{-14}$, $m^3 s^{-1} N^{-1}$	$K_1 \times 10^{-7}$, ms^{-1}
sucrose	0.87	1.16 ± 0.50	0.46 ± 0.09
glycerol	0.50	2.93 ± 0.90	0.57 ± 0.06
1.2-PD	0.50	4.13 ± 0.80	2.88 ± 0.30
Me_2SO	0.61	7.22 ± 0.60	23.10 ± 4.00
EG	0.26	4.13 ± 1.20	25.20 ± 3.00
ethanol	0.30	4.82 ± 0.70	228.00 ± 70.00

Limitations of the study

Our study focuses on a single microalgal species and does not include other taxa, which may limit the generalizability of the findings. In addition, long-term assessments of cell viability and metabolic activity after thawing were not performed. In future work, additional experiments are planned to evaluate these parameters, which will help increase the reliability of the recommendations regarding the optimal incubation time of cells with CPAs prior to freezing.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to N. Chernobai (nadiiachernobai@gmail.com).

Materials availability

This study did not generate new unique materials.

Data and code availability

- All data reported in this article will be shared by the lead contact upon request.
- This article does not report original code.
- Any additional information required to reanalyze the data reported in this article is available from the lead contact upon request.

ACKNOWLEDGMENTS

We would like to thank the Department of Botany at V. N. Karazin Kharkiv National University for their support.

AUTHOR CONTRIBUTIONS

N. Ch., N. Sh., I. K., and L. R. conceived the project. I. K. and L. R. provided supervision and developed the methodology. I. K. performed the microscopy work. N. Ch. and N. Sh. conducted formal analysis, wrote the original draft, and performed review and editing. All authors contributed to article preparation and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Microalgae culture
- METHOD DETAILS
  - CPA and NaCl solutions
  - Study of osmotic reactions
- QUANTIFICATION AND STATISTICAL ANALYSIS

Received: December 19, 2024
Revised: September 1, 2025
Accepted: December 29, 2025
Published: December 31, 2025

REFERENCES

1. Ali, O., Cheddadi, I., Landrein, B., and Long, Y. (2023). Revisiting the relationship between turgor pressure and plant cell growth. New Phytol. *238*, 2217–2232. https://doi.org/10.1111/nph.18683.
2. Gordiyenko, Y.A., and Pushkar, N.S. (1994). Physical Principles of Low Temperature Preservation of Cell Suspensions (Kiev: Naukova dumka).
3. Kedem, O., and Katchalsky, A. (1958). Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. Biochim. Biophys. Acta *27*, 229–246. https://doi.org/10.1016/0006-3002(58)90330-5.
4. Li, L., Chen, Z., Zhang, M., Panhwar, F., Gao, C., Zhao, G., Jin, B., and Ye, B. (2017). Cell membrane permeability coefficients determined by single-step osmotic shift are not applicable for optimization of multi-step addition of cryoprotective agents: As revealed by HepG2 cells. Cryobiology *79*, 82–86. https://doi.org/10.1016/j.cryobiol.2017.09.006.
5. Puhovkin, A.Y., and Kopeika, E.F. (2016). Plasma membrane permeability of carp (Cyprinus carpio, L., 1758) spermatozoa for water and cryoprotectants molecules at different stages of cryopreservation. Problems of Cryobiology and Cryomedicine *26*, 123–134. https://doi.org/10.15407/cryo26.04.340.
6. Day, J.G., and Brand, J.J. (2005). Cryopreservation methods for maintaining microalgal cultures. In Algal Culturing Techniques, R.A. Andersen, ed. (Elsevier Academic Press), pp. 165–187. https://doi.org/10.1016/B978-012088426-1/50013-5.
7. Yang, N.J., and Hinner, M.J. (2015). Getting across the cell membrane: an overview for small molecules, peptides, and proteins. Methods Mol. Biol. *1266*, 29–53. https://doi.org/10.1007/978-1-4939-2272-7_3.
8. Kihika, J.K., Wood, S.A., Rhodes, L., Smith, K.F., Thompson, L., Challenger, S., and Ryan, K.G. (2022). Cryoprotectant treatment tests on three morphologically diverse marine dinoflagellates and the cryopreservation of Breviolum sp. (Symbiodiniaceae). Sci. Rep. *12*, 646.
9. Prieto-Guevara, M., Alarcón-Furnieles, J., Jiménez-Velásquez, C., Hernández-Julio, Y., Espinosa-Araujo, J., and Atencio-García, V. (2023). Cryopreservation of the microalgae Scenedesmus sp. Cells *12*, 156. https://doi.org/10.3390/cells12040562.
10. Tzovenis, I., Triantaphyllidis, G., Naihong, X., Chatzinikolaou, E., Papadopoulou, K., Xouri, G., and Tafas, T. (2004). Cryopreservation of marine microalgae and potential toxicity of cryoprotectants to the primary steps of the aquacultural food chain. Aquaculture *230*, 457–473. https://doi.org/10.1016/S0044-8486(03)00444-7.
11. Boutarfa, S., Senoussi, M.M., Gonzalez-Silvera, D., López-Jiménez, J.Á., and Aboal, M. (2022). The green microalga Coelastrella thermophila var. globulina (Scenedesmaceae, Chlorophyta) isolated from an Algerian hot spring as a potential source of fatty acids. Life *12*, 560. https://doi.org/10.3390/life12040560.
12. Thepsuthammarat, K., Reungsang, A., and Plangklang, P. (2023). Microalga Coelastrella sp. cultivation on unhydrolyzed molasses-based medium towards the optimization of conditions for growth and biomass production under mixotrophic cultivation. Molecules *28*, 3603. https://doi.org/10.3390/molecules28083603.
13. Nobel, P.S. (1969). The Boyle-Van't Hoff relation. J. Theor. Biol. *23*, 375–379. https://doi.org/10.1016/0022-5193(69)90025-3.
14. Joseph, I., Panigrahi, A., and Kishore Chandra, P. (2000). Tolerance of three marine microalgae to cryoprotectants dimethyl sulfoxide, methanol and glycerol. Indian J. Mar. Sci. *29*, 240–243.
15. Crutchfield, A., Diller, K., and Brand, J. (1999). Cryopreservation of Chlamydomonas reinhardtii (Chlorophyta). Eur. J. Phycol. *34*, 43–52. https://doi.org/10.1080/09670269910001736072.
16. Bartolac, L.K., Lowe, J.L., Koustas, G., Grupen, C.G., and Sjöblom, C. (2018). Effect of different penetrating and non-penetrating cryoprotectants and media temperature on the cryosurvival of vitrified *in vitro* produced porcine blastocysts. Anim. Sci. J. *89*, 1230–1239. https://doi.org/10.1111/asj.12996.
17. Li, L., Tian, Y., Li, Z., Duan, P., Wang, X., Chen, S., Wang, L., Liu, Y., Wang, Q., Li, W., et al. (2023). Effect of non-permeable cryoprotectant sucrose on the development of spotted knifejaw (Oplegnathus punctatus) embryos. Cryobiology *112*, 104555. https://doi.org/10.1016/j.cryobiol.2023.104555.
18. Yong, K.W., Laouar, L., Elliott, J.A.W., and Jomha, N.M. (2020). Review of non-permeating cryoprotectants as supplements for vitrification of mammalian tissues. Cryobiology *96*, 1–11. https://doi.org/10.1016/j.cryobiol.2020.08.012.
19. Paredes, E., Ward, A., Probert, I., Gouhier, L., and Campbell, C.N. (2021). Cryopreservation of algae. In Cryopreservation and Freeze-Drying Protocols. Methods in Molecular Biology, J.G. Day, ed. (Humana Press), pp. 1–20. https://doi.org/10.1385/0896032965.
20. Reinders, A., Sivitz, A.B., and Ward, J.M. (2012). Evolution of plant sucrose uptake transporter. Front. Plant Sci. *3*, 22. https://doi.org/10.3389/fpls.2012.00022.
21. Gutknecht, J. (1968). Permeability of Valonia to water and solutes: Apparent absence of aqueous membrane pores. Biochim. Biophys. Acta *163*, 20–29. https://doi.org/10.1016/0005-2736(68)90028-X.
22. Walsh, J.R., Diller, K.R., and Brand, J.J. (2004). Measurement and simulation of water and methanol transport in algal cells. J. Biomech. Eng. *126*, 167–179. https://doi.org/10.1115/1.1688775.
23. Moisieiev, A., Kovalenko, I., Kovalenko, S., Bozhok, G., and Gordienko, O. (2021). Dynamics of dimethyl sulfoxide penetration into L929 cells and

L929-based spheroids. Probl. Cryobiol. Cryomed. *31*, 316–325. https://doi.org/10.15407/cryo31.04.316.

24. Rippka, R., Deruelles, J., and Waterbury, J.B. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. *111*, 1–61. https://doi.org/10.1099/00221287-111-1-1.
25. Allen, M.M., and Stanier, R.Y. (1968). Selective isolation of blue-green algae from water and soil. J. Gen. Microbiol. *51*, 203–209. https://doi.org/10.1099/00221287-51-2-203.
26. Ortega, J.K.E. (2023). Theoretical analyses of turgor pressure during stress relaxation and water uptake, and after changes in expansive growth rate when water uptake is normal and reduced. Plants *12*, 1891. https://doi.org/10.3390/plants12091891.
27. Silva, H.R.d., Silva, F.C.P.d., Prete, C.E.C., Hoshino, R.T., Faria, R.T.d., Mantovani, M.S., and Guedes, C.L.B. (2020). Cryopreservation of Chlorella vulgaris using different cryoprotectant agents. J. Agric. Sci. *12*, 75–163. https://doi.org/10.5539/jas.v12n7p75.
28. Yuan, S.Y., and Rigor, R.R. (2011). Regulation of endothelial barrier function. Colloquium Series on Integrated Systems Physiology: From Molecule to Function *3*, 1–146. https://doi.org/10.4199/C00025ED1V01Y201101ISP013.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
The culture of Coelastrella thermophila var. глобулин	Department of Botany of V.N. Karazin Kharkiv National University	N/A
Chemicals, peptides, and recombinant proteins		
1,2-Propanediol	https://www.sigmaaldrich.com/HR/en/product/sial/398039?icid=sharepdp-clipboard-copy-productdetailpage	Sigma-Aldrich 398039 1,2-Propanediol
glycerol	https://www.sigmaaldrich.com/HR/en/product/sigald/g7893?icid=sharepdp-clipboard-copy-productdetailpage	Sigma-Aldrich G7893 Glycerol
ethylene glycol	https://www.sigmaaldrich.com/HR/en/product/sial/324558?icid=sharepdp-clipboard-copy-productdetailpage	Sigma-Aldrich 324558 Ethylene glycol
dimethyl sulfoxide	https://www.sigmaaldrich.com/HR/en/product/sigald/472301?icid=sharepdp-clipboard-copy-productdetailpage	Sigma-Aldrich 472301 Dimethyl sulfoxide
sucrose	https://www.sigmaaldrich.com/HR/en/product/sial/s5016?icid=sharepdp-clipboard-copy-productdetailpage	Sigma-Aldrich S5016 Sucrose
Software and algorithms		
modified Kedem-Katchalsky model	Kedem et al.[3]	N/A

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Microalgae culture

The culture of *C. thermophila var. globulina* was obtained from the collection of microalgae of the Department of Botany of V.N. Karazin Kharkiv National University. Microalgae cells were cultured on BG-11 nutrient medium[24] at 25±2°C without aeration. Biomass accumulation was performed in 40 ml culture flasks (TPP, Switzerland), under day-and-night illumination with white fluorescent light 52.84 μmol photons $m^{-2}s^{-1}$ (3kLux).[25] The growth stage was determined from the growth curve by counting the number of cells (cells/mL) on days 3, 7, 10, 14, 21, 28, and 35 of cultivation.

METHOD DETAILS

CPA and NaCl solutions

Cell permeability coefficients have been studied for a range of substances used as CPAs or included in cryoprotective mixtures for microalgae and plant cells cryopreservation: 1,2-propanediol (1,2-PD), glycerol, ethylene glycol (EG), dimethyl sulfoxide (Me_2SO), ethanol, sucrose. The concentration of all CPAs came to 3.4 M (the final concentration was 1.7 M), except for sucrose, which was 1 M (the final concentration was 0.5 M). It is at these concentrations that the substances under study are included in the composition of cryoprotectant mixtures (plant vitrification solutions) used for the cryopreservation of microalgae and plants.[17,26,27]

Due to the low osmolarity of the microalgae cultural medium (less than 0.1 M (0.2 Osmol/L)) to determine the value of osmotically inactive fraction, we studied the osmotic behavior of *C. thermophila var. globulina* cells in hypertonic NaCl solutions of different concentrations that range from 1 M to 3.4 M (from 2 to 6.8 Osmol/L) (the final concentration was from 0.5 M to 1.7 M).

Solutions of NaCl and CPAs were prepared in BG-11 medium.

Study of osmotic reactions

The assessment of cell mass transfer processes was conducted using a volumetric method, comparing the experimentally obtained dependencies of the cell volume change from the time of their exposition to CPA solutions in the BG-11 medium, and curves that are solutions of the modified Kedem-Katchalsky model.[2] Normalized cell volume $V(t)/V_0$ (where V(t) was time dependent cell volume

change, V_0 was the initial cell fraction) was determined by measuring the diameter of cells on photographs obtained with a microscope Axio Observer Z1 (CarlZeiss, Germany). The photographs were processed with AxioVision 4.8 (Carl Zeiss). The measurements were conducted at 25 °C.

The microalgae culture was studied during the mid-exponential growth phase (21st day of cultivation), which allowed for the collection of cells of almost uniform size, and no additional fixation on the microscope slide was required during the experiment. The sample was placed on a slide with a cavity, and the tested cryoprotectant was added directly to the drop under the microscope objective in a 1:1 ratio. The cell size fixation was performed as quickly as possible, but no longer than 3 seconds. The change in cell volume was taken as the average in the microscope's field of view and compared to the control.

The contact of cells with a solution of penetrating substances, such as a solution of penetrating CPAs, is accompanied with time dependent changes of the cell volume (V) and of concentrations of solutes outside and inside the cells and could be described by the system of differential equations[2]:

$$\begin{aligned}\frac{d}{dt}V &= SL_p\left[\sigma_1\left(\pi_1^{in}-\pi_1^{out}\right)+\pi_2^{in}-\pi_2^{out}-\Delta p\right]\\ \frac{d}{dt}\pi_1^{in} &= -\left[SK_1\left(\pi_1^{in}-\pi_1^{out}\right)-\sigma_1\pi_1^{in}\frac{dV}{dt}\right]\frac{1}{V-\alpha V_0}\end{aligned} \quad \text{(Equation 1)}$$

$$\pi_2^{in} = \pi_{20}^{in}\frac{1-\alpha}{(V/V_0-\alpha)} \quad \text{(Equation 2)}$$

where t – time; S and Lp – surface area and filtration coefficient of membrane; π_1^{out} and π_1^{in} – osmotic pressures of substance penetrating across through plasma membrane substance outside and inside the cell, respectively; π_2^{out} and π_2^{in} – total osmotic pressures of the substance non-penetrating through the plasma membrane outside and inside the cell, respectively; Δp – hydrostatic pressure differential on cell membrane; K_1 and σ_1 – coefficients of permeability and reflection of the plasma membrane for the substance penetrating through the membrane, respectively; π_{20}^{in} and V_0 – initial values of total osmotic pressure of non-penetrating substances inside a cell and its initial volume, respectively; α – fraction of osmotically inactive intracellular substances, which was defined as the minimum volume in concentrated solutions of non-penetrating substances. It is assumed that the difference in hydrostatic pressure on the cell membrane under these experimental conditions does not occur, so $\Delta p = 0$.

The transition to dimensionless quantities brings the Equation 1 to the form:

$$\frac{dy}{dt} = \frac{1}{\tau_0}\left[\sigma_1\left(\hat{\pi}_1^{in}-\hat{\pi}_1^{out}\right)+\frac{1-\alpha}{y-\alpha}-1\right] \quad \text{(Equation 3)}$$

$$\frac{d\hat{\pi}_1^{out}}{dt} = -\frac{1}{\tau_1}\left[\left(\hat{\pi}_1^{in}-\hat{\pi}_1^{out}\right)+\sigma_1\hat{\pi}_1^{in}\frac{dy}{dt}\right]\frac{1}{y-\alpha}\hat{\pi}_2^{in} = \hat{\pi}_{20}^{in}\left(\frac{1-\alpha}{y-\alpha}\right) \quad \text{(Equation 4)}$$

where $y = \frac{V}{V_0}$ – relative cell volume, $\tau_0 = \frac{1}{L_p\gamma\hat{\pi}_0^{in}}$; $\tau_1 = \frac{1}{K_i\gamma}$; π_{20}^{in} = 200 mOsm/l, $\hat{\pi}_{1,2}^{in,out} = \pi_{1,2}^{in,out}/\pi_{20}^{in}$ – normalized osmotic pressure of penetrating (1) and non-penetrating (2) components inside and outside the cell; γ – surface-volume ratio of a cell:

$$\gamma = \frac{S_0}{V_0} = \frac{4\pi r^2}{4/3\,\pi r^3} = \frac{3}{r} = \frac{6}{d} \quad \text{(Equation 5)}$$

where *d* – initial diameter of a cell, measured experimentally.

The reflection coefficient of the cell membrane (σ_1) is a measure of its permeability to various substances, it is ranges from 0 to 1, where 0 indicates complete permeability of the membrane to a substance, and 1 indicates complete absence of penetration of the substance through the membrane. The reflection coefficient considers the limitations on solute permeability based on the solute's molecular size in comparison to the average pore size of the permeability pathway. Larger molecules face greater resistance and are more often "reflected" by the membrane, resulting in a higher σ_1 value. In contrast, smaller molecules are less frequently reflected, leading to a lower σ_1 value. Based on the sizes of the molecules of CPAs that we were used, the σ_1 took values of 0.5 (for ethanol and ethylene glycol molecules), 0.95 (for 1,2-PD, glycerol, Me_2SO and sucrose molecules) and 1 (for NaCl molecules).[28]

The coefficients of cell membrane permeability were determined by solving differential Equation 2. By adjusting the parameters τ_0 and τ_1, we were able to find the values of Lp and K_1 by matching the theoretical curves to the experimental data.

QUANTIFICATION AND STATISTICAL ANALYSIS

For the investigation of each cell property (*e.g.* the membrane permeability to Me_2SO) a total of 15 cells were used per CPA solution. Statistical analysis was conducted using the Student's t-test. The results are expressed as the mean ± standard deviation, with a P-value of less than 0.05 considered statistically significant.