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Effect of polymerization of zwitterionic monomers on their intrinsic antifreeze activity

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We synthesized amino acid–based zwitterionic monomers and investigated their ice recrystallization inhibition (IRI) activity. Among the materials examined, a serine-based zwitterionic acrylate exhibited superior IRI activity. Interestingly, the IRI activity of this zwitterionic acrylate was reduced after polymerization.

Antifreeze materials that control the shape and growth of ice have a high demand across various fields.[1–3] Specifically, in the medical field, antifreeze materials can protect biological samples from physical damage caused by ice recrystallization during cryopreservation, thereby improving cell recovery rates.[4,5] Organisms inhabiting cold regions express antifreeze proteins (AFPs) to protect their cells and tissues from freezing damage at subzero temperatures.[6] Although AFPs have been actively studied for their potential applications, the use of natural AFPs faces challenges such as stability issues and high costs associated with extraction and purification. Considering potential toxicity of molecular cryoprotectants like dimethyl sulfoxide (DMSO), which are commonly used for cell preservation, synthetic polymer-based AFP mimics have been explored as alternative materials.[7–9] These polymers offer advantages such as lower cost and the inability to permeate cell membranes, making them promising candidates for cryoprotection and ice control applications. However, discovering polymeric materials that fully replicate the antifreeze property and cryoprotective activity of natural AFPs is challenging, and the design principles for such materials remain unclear.

Ice recrystallization inhibition (IRI) activity is one of the indicators used to evaluate the performance of antifreeze materials.[10,11] Peptides that mimic antifreeze protein sequences have been reported to exhibit IRI activity.[12] Among synthetic polymers, Gibson and co-workers reported polyvinyl alcohol (PVA) as a material exhibiting exceptionally high IRI activity.[13] The IRI activity of PVA increases with molecular weight, and PVA_{351} can exhibit its IRI activity at a low concentration of 0.05 g L^{-1}. Although its IRI activity is lower than that of natural AFPs, it remains the highest among synthetic polymers. Additionally, Gibson and co-workers recently reported that phenylalanine, a hydrophobic amino acid, and its derivatives exhibit IRI activity despite being low-molecular-weight compounds.[14] Phenylalanine contains both hydrophobic ($-C_6H_6$) and charged moieties ($-NH_3^+$ and $-COO^-$) within its molecular structure, and the coexistence of these features has been suggested to be essential for the expression of IRI activity. However, zwitterionic polymers bearing charged moieties in their side chains and hydrophobic main chains do not universally exhibit IRI activity, and the observed outcome is case-dependent.[9,15] Comprehensive understanding in this field is still lacking.

In this study, we synthesized zwitterionic polymers from zwitterionic monomers that exhibit IRI activity and investigated the effects of polymeric structures on their IRI activity. As zwitterionic monomers, we synthesized amino acid-based monomers with distinct hydrophobicity based on the acrylic moieties and alkyl chains; L-serine methacrylate (SerMA), L-serine acrylate (SerAc), L-2,3-diaminopropanoic acid acrylamide (ApaAm), and L-ornithine acrylamide (OrnAm) (Fig. 1 and Fig. S1–S4). The four monomers derived from α-amino acids can exist as zwitterions in aqueous solution. The IRI activity of the synthesized monomers was evaluated using the splat-cooling assay.[10,11] A 10 μL aliquot of a monomer solution in a 10 mM phosphate-buffered saline (PBS) was dropped from a height of 1.4 m onto a glass coverslip precooled to −78 °C. The resulting thin ice wafer was transferred to a cooling stage and incubated at −8 °C for 30 min to allow recrystallization. The mean largest grain size (MLGS) of the ice crystals after recrystallization was measured (μm) and compared with that of a PBS control, with MLGS (%) calculated as the ratio ($n = 10$). Phenylalanine, used as a positive control, exhibited low MLGS values (5.7–21.5%) at concentrations of 5–20 g L^{-1}, consistent with previous studies (Fig. 2 and Fig. S14).[16] In contrast, L-serine, which possesses a polar moiety but lacks a hydrophobic moiety, exhibited high MLGS values (71.5–78.6%) across all

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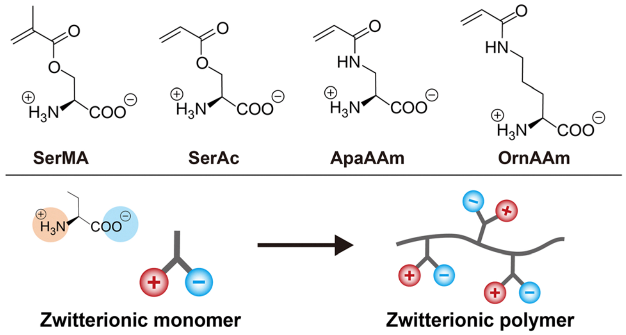

Fig. 1 Chemical structures of polymerizable zwitterionic monomers in the current study and schematic illustration of the polymer synthesis.

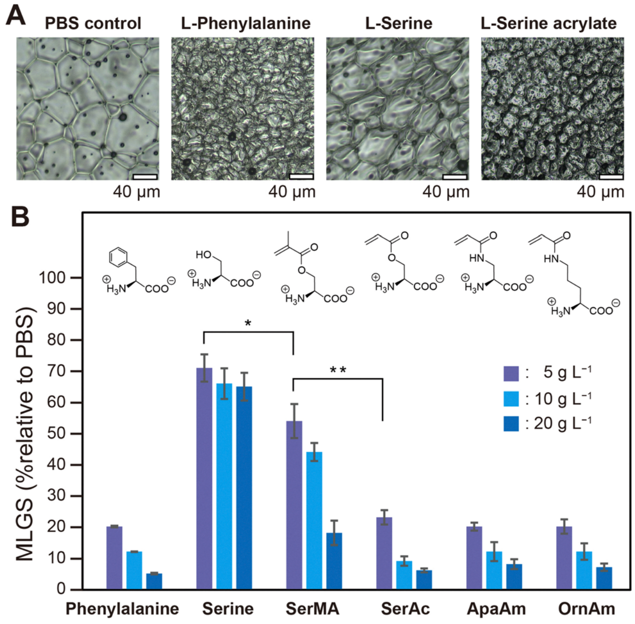

Fig. 2 Ice recrystallization inhibition activity of the compounds evaluated by the splat-cooling assay. (A) Representative micrographs of ice crystals formed in the presence of L-phenylalanine, L-serine, and L-serine acrylate (SerAc) dissolved in PBS at 5 g L^{-1}. Scale bars represent 40 μm. (B) Mean largest grain size (MLGS) of each compound relative to a PBS control, expressed as a percentage. Error bars represent the standard deviation from at least three measurements. Statistical significance was determined by one-way ANOVA (*$P < 0.05$ and **$P < 0.01$).

concentrations, indicating negligible IRI activity. Interestingly, the synthesized zwitterionic monomers SerAc, ApaAm, and OrnAm exhibited IRI activity comparable to that of phenylalanine. These findings suggest that acrylic moieties of the monomers are more favorable for IRI activity than the hydroxyl group of L-serine. The different length of alkyl-chains of ApaAm and OrnAm did not provide difference in their IRI activity. Relatively low IRI activity of SerMA is likely due to its low solubility in PBS even at 5 g L^{-1}.

We then aimed to enhance IRI activity by polymerizing SerAc, which exhibited the highest IRI activity among the monomers. We synthesized the zwitterionic polymer of SerAc *via* oxygen-tolerant photo-reversible addition–fragmentation chain transfer (RAFT) polymerization, a type of living radical polymerization (Table S1 and Fig. S6–S8).[17–19] Three types of SerAc polymers with distinct molecular weights were obtained (SerAc$_{20}$, SerAc$_{85}$ and SerAc$_{128}$). Surprisingly, both SerAc$_{85}$ and SerAc$_{128}$ showed suppressed IRI activity at concentrations of 10–20 g L^{-1} (Fig. 3 and Fig. S14). Furthermore, the shortest polymer, SerAc$_{20}$, exhibited MLGS values comparable to a negative control (polyethylene glycol, PEG) at 5–20 g L^{-1}, indicating a complete lack of IRI activity of the zwitterionic groups. Dynamic light scattering measurements showed no aggregation behaviors of the polymers in PBS (Fig. S13). These results indicate that polymerization of SerAc led to decrease in intrinsic IRI activity. It is noteworthy that SerAc$_{85}$ and SerAc$_{128}$ still retained superior IRI activity compared to PEG.

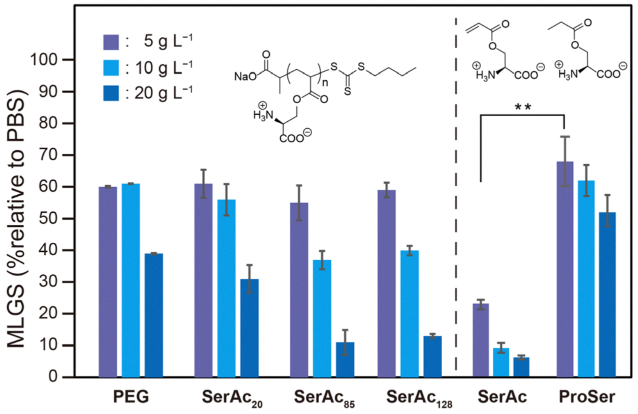

Fig. 3 Ice recrystallization inhibition activity of the compounds evaluated by the splat-cooling assay. Mean largest grain size (MLGS) of each compound relative to a PBS control, expressed as a percentage. Error bars represent the standard deviation from at least three measurements. Statistical significance was determined by one-way ANOVA (**$P < 0.01$).

We hypothesized two possible reasons for the decrease in IRI activity of SerAc upon polymerization: (i) electrostatic interactions between neighboring zwitterionic moieties along the polymer chain due to their close proximity, and (ii) the crucial role of the vinyl group in the monomer for ice interactions, which is lost upon polymerization. To suppress intramolecular electrostatic interactions, nonionic monomers were randomly incorporated into the zwitterionic polymer. Methyl acrylate (MA), methoxyethyl acrylate (MEA), hydroxyethyl acrylate (HEA), and *N*,*N*-dimethylacrylamide (DMA) were copolymerized with SerAc. The target copolymerization ratio was 50 mol%, resulting in random copolymers–SerAc$_{29}$MA$_{11}$, SerAc$_{25}$MEA$_{27}$, SerAc$_{28}$HEA$_{37}$, and SerAc$_{26}$DMA$_{24}$–each containing comparable amounts of zwitterionic groups to SerAc$_{20}$ (Table S2 and Fig. S9–12). The synthesized copolymers exhibited MLGS values in the range of 36–53% at 10 g L^{-1} and 15–44% at 20 g L^{-1} (Fig. S15 and S16). Although slight differences in MLGS values were observed depending on the structure of the nonionic comonomers, copolymerization did not result in a significant improvement in IRI activity compared with the SerAc homopolymer.

Next, for comparison with SerAc, we synthesized L-propionyl serine (ProSer), a zwitterionic compound lacking a vinyl group, and evaluated its IRI activity (Fig. S5). At concentrations of 5–10 g L^{-1}, ProSer did not exhibit any inherent IRI activity

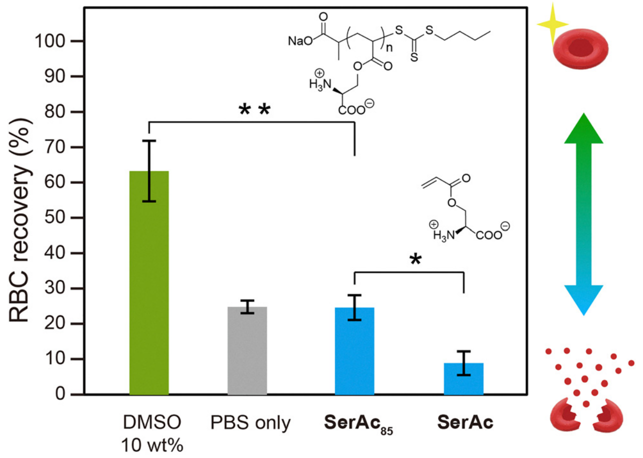

Fig. 4 Cryoprotection of sheep red blood cells using a 10 wt% DMSO solution, a SerAc solution (10 g L^{-1} in PBS), and a $SerAc_{85}$ solution (10 g L^{-1} in PBS). Cell viability after a single freeze–thaw cycle is expressed as a percentage. The experiments were triplicated. Statistical significance was determined by one-way ANOVA ($*P < 0.05$ and $**P < 0.01$).

(Fig. 3 and Fig. S14). The pronounced difference in IRI activity observed between SerAc and ProSer suggests that the presence of the vinyl group plays a crucial role in the high IRI activity of SerAc. To elucidate the differences in the IRI activity in terms of the hydration structures of the zwitterionic molecules, we conducted molecular dynamics (MD) simulations (Table S3 and Fig. S17–20).[20] The three types of zwitterions have the effect of breaking the hydrogen bonds between water molecules and tendency to accelerate the dynamics of water. However, phenylalanine, SerAc, and ProSer exhibited similar hydration structures in the simulation at 300 K, likely due to the dominant contribution of the zwitterionic moiety to hydration under these conditions. To further clarify the relationship between molecular structure and IRI activities, MD simulations under low-temperature conditions are likely required.[16,21–23] Based on these findings, we conclude that the loss of IRI activity upon polymerization is attributed to the absence of the vinyl group, which disrupts the hydrophilic–hydrophobic balance or electron-rich structural features that are essential for ice-binding interactions.

Finally, we evaluated cryoprotective effects of the synthesized SerAc-based polymers using sheep red blood cells (RBCs). The following solutions were added to cryopreservation vials together with 500 μL of the RBC suspension: a 10 wt% DMSO solution, SerAc solutions (10 g L^{-1} in PBS), and $SerAc_{85}$ solutions (10 g L^{-1} in PBS). After a single freeze–thaw cycle, hemoglobin leakage was quantified to assess the extent of freezing-induced RBC damage, and the RBC protection efficiency (RBC recovery) was calculated (Fig. 4). The recovery values for SerAc and $SerAc_{85}$ were consistently low (7.8% to 24.1%) compared with PBS alone (24.4%). These results indicate that the synthesized zwitterionic monomers and polymers did not exhibit cryoprotective effects toward RBCs. In contrast, other zwitterionic polymers, such as poly(carboxybetaine methacrylate) (PolyCBMA) and poly(sulfobetaine methacrylate) (PolySBMA), have been reported to show excellent cryoprotective effects on chondrocytes and are being investigated as alternatives to DMSO-based cryoprotectants.[24–26]

In conclusion, we synthesized zwitterionic monomers derived from amino acids and demonstrated that these monomers exhibited IRI activity comparable to that of phenylalanine, which is known for its high antifreeze activity. We further prepared homopolymer and random copolymers incorporating zwitterionic units. Although polySerAc exhibited reduced IRI activity compared with its monomeric counterpart, it still retained IRI activity. Control experiments revealed that the loss of the vinyl group of SerAc was a key factor contributing to the decrease in IRI activity. Furthermore, PolySerAc did not exhibit cryoprotective effects toward RBCs, in contrast to other zwitterionic polymers that have been reported to possess cryoprotective properties. These findings of the current study contribute to elucidate the mechanisms of antifreeze materials incorporating zwitterionic moieties.

Author contributions

M. N. planned the project and wrote the manuscript. S. T. and H. Y. conducted the experiments and wrote the manuscript. Y. H. conducted MD simulation and analysed the results. Y. M. and H. M. provided advice to improve the quality of the research.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: details of the experimental procedure, list of materials, ^1H NMR spectra of the compounds, and results of IRI assay. See DOI: https://doi.org/10.1039/d6ma00147e.

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References

1 N. S. Ustun and S. Turhan, *J. Food Process. Preserv*, 2015, **39**, 3189–3197.
2 Z. He, K. Liu and J. Wang, *Acc. Chem. Res.*, 2018, **51**, 1082–1091.
3 Y. Gao, H. Qi and L. Zhang, *Ind. Eng. Chem. Res.*, 2023, **62**, 7839–7858.
4 Z. Liu, X. Zheng and J. Wang, *J. Am. Chem. Soc.*, 2022, **144**, 5685–5701.
5 T. Chang and G. Zhao, *Adv. Sci.*, 2021, **8**, 2002425.
6 G. Gharib, S. Saeidiharzand, A. K. Sadaghiani and A. Koşar, *Front. Bioeng. Biotechnol.*, 2022, **9**, 770588.

7 M. Gore, A. Narvekar, A. Bhagwat, R. Jain and P. Dandekar, *J. Mater. Chem. B*, 2022, **10**, 143–169.

8 C. Stubbs, T. L. Bailey, K. Murray and M. I. Gibson, *Biomacromolecules*, 2020, **21**, 7–17.

9 T. Ishibe, N. Gonzalez-Martinez, P. G. Georgiou, K. A. Murray and M. I. Gibson, *ACS Polym. Au*, 2022, **2**, 449–457.

10 C. A. Knight, J. Hallett and A. L. DeVries, *Cryobiology*, 1988, **25**, 55–60.

11 S. Abraham, K. Keillor, C. J. Capicciotti, G. E. Perly-Robetson, J. W. Keillor and R. N. Ben, *Cryst. Growth Des.*, 2015, **15**, 5034–5039.

12 M. Kaganovich, E. Gibeon, A. S. Bakalinsky, D. E. Shalev, I. Braslavsky and M. Reches, *ACS Appl. Mater. Interfaces*, 2025, **17**, 16256–16267.

13 T. Congdon, R. Notman and M. I. Gibson, *Biomacromolecules*, 2013, **14**, 1578–1586.

14 M. T. Warren, I. Galpin, M. Hasan, S. A. Hindmarsh, J. D. Padrnos, C. Edwards-Gayle, R. T. Mathers, D. J. Adams, G. C. Sosso and M. I. Gibson, *Chem. Commun.*, 2022, **58**, 7658–7661.

15 D. E. Mitchell, M. Lilliman, S. G. Spain and M. I. Gibson, *Biomater. Sci.*, 2014, **2**, 1787–1795.

16 J. Ma, X. Zhang, Z. Cui, M. Zhao, L. Zhang and H. Qi, *J. Mater. Chem. B*, 2023, **11**, 4042–4049.

17 Y. Lee, C. Boyer and M. S. Kwon, *Chem. Soc. Rev.*, 2023, **52**, 3055–3097.

18 C. Stubbs, K. A. Murray, T. Ishibe, R. T. Mathers and M. I. Gibson, *ACS Macro Lett.*, 2020, **9**, 290–294.

19 M. Nagao, S. Tanaka and Y. Miura, *Mater. Adv.*, 2023, **4**, 3192–3196.

20 Y. Higuchi, M. A. Saleh, T. Anada, M. Tanaka and M. Hishida, *J. Phys. Chem. B*, 2024, **128**, 5008–5017.

21 L. Weng, S. L. Stott and M. Toner, *Langmuir*, 2018, **34**, 5116–5123.

22 M. T. Warren, I. Galpin, F. Bachtiger, M. I. Gibson and G. C. Sosso, *J. Phys. Chem. Lett.*, 2022, **13**, 2237–2244.

23 K. Mochizuki and V. Molinero, *J. Am. Chem. Soc.*, 2018, **140**, 4803–4811.

24 M. Liu, X. Zhang, H. Guo, Y. Zhu, C. Wen, X. Sui, J. Yang and L. Zhang, *Biomacromolecules*, 2019, **20**, 3980–3988.

25 R. Rajan, F. Hayashi, T. Nagashima and K. Matsumura, *Biomacromolecules*, 2016, **17**, 1882–1893.

26 Y. Sun, D. Maltseva, J. Liu, T. Hooker II, V. Mailänder, H. Ramløv, A. L. DeVries, M. Bonn and K. Meister, *Biomacromolecules*, 2022, **23**, 1214–1220.