

Effect of ion structure on cellulase

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Ionic liquids have attracted significant attention as biomass solvents. However, their high toxicity limits their practical application. In contrast, cellulose-dissolving zwitterions have lower toxicity and are more suitable for biomass processing. Although zwitterions with longer alkyl spacers (Fig. 1, b) show superior cellulose solubility, they significantly inhibit cellulase activity, and the mechanism of this inhibition remains unclear.

In this study, the effects of zwitterions with various alkyl chains (Fig. 1) on cellulase activity were investigated. The zwitterionic structure reduced inhibition compared to [C₂mim]OAc. Increasing the carbon number of alkyl chains (Fig. 1 a and b) enhanced the inhibitory effect regardless of the place. Introducing an oligoether chain reduced the inhibition. The inhibition by zwitterions was classified into 3 types: pH change, reversible inhibition, and irreversible inhibition (Fig. 2). The effect of pH was similar in all used zwitterions. Reversible inhibition was the main factor of the inhibition in the case of short-alkyl-chain zwitterions. In contrast, [C₂mim]OAc and zwitterions with long alkyl chains caused strong irreversible inhibition, mainly due to enzyme denaturation. The introduction of oligoether groups effectively prevented this denaturation.

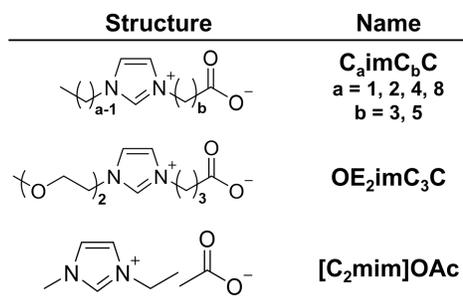


Fig. 1. Zwitterions (ZIs) and an ionic liquid (IL) used in this study.

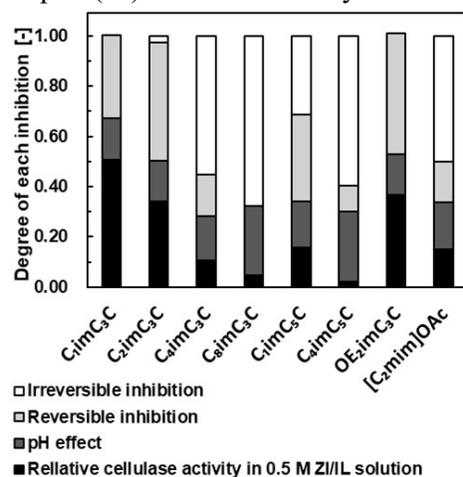


Fig. 2 The ratio of categories of cellulase inhibition by zwitterions.

References

1. K. Kuroda *et al.*, *J. Am. Chem. Soc.*, 2017, **139**, 16052–16055
2. H. Tobe *et al.*, *Ind. Eng. Chem. Res.*, 2024, **63**, 12297–12306



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I am a master course student at the Graduate School of Natural Science and Technology, Kanazawa University, Japan. I received Bachelor of Engineering from Kanazawa University in 2024. My research focuses on the effects of ionic and zwitterionic liquids on cellulase activity for efficient biomass conversion. In 2024, I presented my findings at the Ionic Liquid Research Association in Japan.