Preparation of a Salt-Responsively Soluble-Insoluble Enzyme and Its Application to Hydrolysis of Biomass

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Summary

A copolymer of methacrylic acid (MAA) and N-isopropyl acrylamide (NIPAM) was used as a novel reversibly soluble-insoluble support whose solubility changes depending on the NaCl concentration of the solution at a constant temperature. Amylase (Dabiase K-27) immobilized covalently on the salt-responsive polymer showed good response of solubility : The immobilized enzyme (D-MN) was in a soluble state below 0.5% NaCl, but in insoluble form above 1.0% NaCl at 30 ℃. D- MN in a soluble state has a high specific activity for hydrolysis of uncooked starch. When more than 2.0% NaCl was added to a buffer solution (pH 4.5) with D-MN at 30 ℃, the activity of D-MN for uncooked starch was lower than that in the buffer solution without NaCl. As the NaCl concentration of the buffer solution became high, the activity of D-MN decreased gradually. The activity of D-MN was repressed completely by adding NaCl to the buffer solution at 5%.

On the basis of these results, we tried to regulate the activity of D-MN by changes of NaCl concentration during hydrolysis reaction of uncooked starch. At 3 hr of a reaction time, by adding NaCl to a reaction medium at 5%, the hydrolysis was stopped. After removing NaCl from the reaction medium by dialysis, D-MN could be used successively for repeated hydrolysis reactions of uncooked starches, in which D-MN was insolubilized by adjusting the NaCl concentration of a reaction mixture at 30 ℃ from 0% to 5%.