

## 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 as well as the temperature of the solution. Amylase (Dabiase K-27) immobilized covalently on the salt- and thermo-responsive polymer showed good response of solubility : The immobilized enzyme (D-MN) was in a soluble state below 32°C, but in insoluble form above 42°C. D-MN in a soluble state has a high specific activity for hydrolysis of soluble starch or uncooked starch. Adding 0.5% NaCl to a buffer solution (pH 4.5) with D-MN, the solubility response of D-NM to changes in the temperature of the solution was more sensitive than that in the buffer solution without NaCl. D-MN was used successively for repeated hydrolysis reactions of soluble and uncooked starches, in which D-MN was insolubilized by adjusting either the temperature of a reaction mixture with 0.5% NaCl from 30°C to 36°C or the NaCl concentration of a reaction mixture at 30°C from 0% to 1%. In the repeated hydrolysis, glucose was produced successively from soluble and uncooked starches and D-MN can be repeatedly used after recovering from a reaction product by centrifugation at the end of each batchwise hydrolysis.