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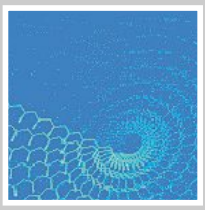

Polymer and Colloid Highlights

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ATRPases: Enzymes as Catalysts for Atom Transfer Radical Polymerization

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Enzymes have proven to be useful catalysts in the toolbox of synthetic chemists because they work under mild conditions both in water and in organic solvents and display high regio-, stereo- and substrate selectivity. Nature's catalysts have been extensively studied as catalysts for polymerization reactions such as polycondensations, ring-opening polymerizations, and free radical polymerizations.[1,2] However, enzymatic activity in controlled radical polymerizations, such as atom transfer radical polymerization (ATRP), was unknown until we discovered that the heme enzyme horseradish peroxidase (HRP) catalyzes polymerizations under the reductive conditions of activators regenerated by electron transfer (ARGET) ATRP.[3] We refer to enzymes that possess this novel activity as ATRPases.

Conventional catalysts for ATRP are based on transition metal complexes,[4] which are (mildly) toxic, environmentally unfavorable and can contaminate the products.[5] This hampers the application of ATRP-derived polymers, *e.g.* in biomedical devices and food packaging. Because enzymes are environmentally friendly, non-toxic, and easy to remove from polymer solutions, ATRPases could become 'green' alternatives to metal complex catalysts.

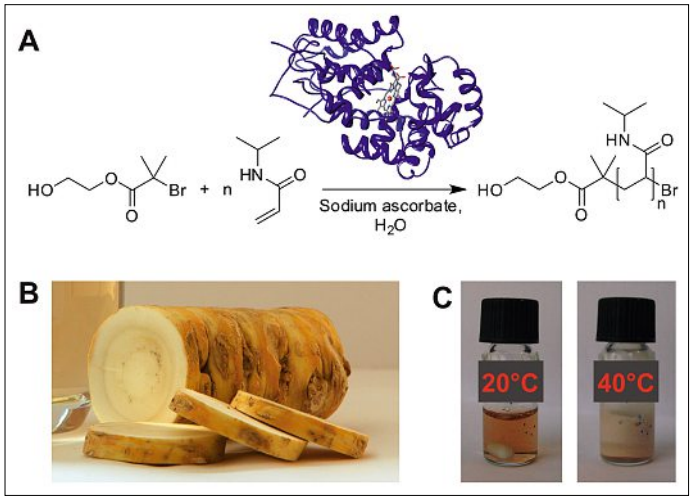

Fig. 1. A) Horseradish peroxidase-catalyzed polymerization of NIPAAm under ARGET ATRP conditions. B) The source of the enzyme is the root of horseradish *(Amoracia rusticana).* C) Reaction mixture after polymerization showing the brown color of the peroxidase and the characteristic precipitate of polyNIPAAm at elevated temperatures.

HRP catalyzed the polymerization of *N*-isopropyl acrylamide (NIPAAm) using an alkylbromide initiator and sodium ascorbate as the reducing agent in aqueous buffers (Fig. 1). No peroxides, the native substrates of HRP, were needed for this reaction. The polymerization resulted in polymers with relatively narrow, monomodal molecular weight distributions (polydispersity indices ≥1.44). Moreover, polymers with bromine chain ends were obtained, as determined by neutron activation analysis. These findings indicate that the enzyme exhibits control of polymerization and that the mechanism involves reversible bromine atom transfer from the initiator to the enzyme and back to the growing polymer radical in an ATRP-type equilibrium.

A multi-parameter space is available to optimize the catalytic performance of enzymes in ATRP. For example, a characteristic feature of any enzymatic activity is its dependency on the protonation state of the biomolecule and thus on the pH of the reaction solution. Indeed, the pH influenced several aspects of the polymerization, *e.g.* conversion and molecular weight distribution, with an optimum at pH 6–7.

HRP remained stable under the reaction conditions. UV/Vis and circular dichroism spectroscopy revealed no significant differences between spectra of the enzyme recorded after polymerization to those recorded prior to polymerization. Moreover, gel electrophoresis and mass spectrometry showed that the polymer radicals did not attack the polypeptide, which otherwise would have led to the formation of polymer–protein conjugates.

In conclusion, biocatalytic ATRP is a promising new approach to environmental friendly synthesis of polymers and represents non-explored enzymatic activity. A multitude of possibilities, *e.g.* the use of other metalloenzymes and genetic engineering methods, will be explored in future work in order to enhance the catalytic performance of ATRPases.

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