Polyphosphate Kinase – Enzyme Stabilization Through Immobilization

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Introduction

Aim: Enzyme stabilization by immobilization for the integration in a cytidine triphosphate (CTP) regeneration cascade in a continuous packed bed reactor

- The NDP polyphosphate phosphotransferase 3 from *Ruegeria pomeroyi* (RpPPK2-3, UniProt KB: Q5LSN8) belongs to the polyphosphate kinase (PPK) family 2, class 1 subfamily (Fig. 1). [1]
- The RpPPK2-3 converts nucleotide diphosphates (NDPs) and polyphosphate (PolyP) to nucleotide triphosphates (NTPs) (Fig. 2). [2]

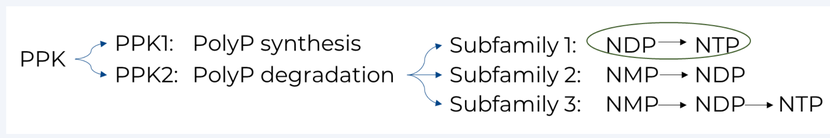

Fig. 1: Polyphosphate kinase (PPK) family

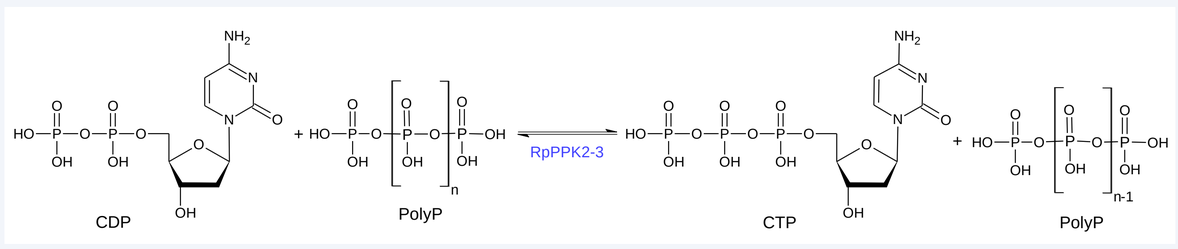

Fig. 2: Conversion of CDP and PolyP to CTP with RpPPK2-3

- Continuous biocatalytic processes are becoming increasingly important and for this long-term stable and reusable enzymes are required.
- Immobilisation can increase enzyme stability and can influence specificity, selectivity and potentially reduce inhibitions. [3]
- Covalent carrier binding offers among various immobilization methods the most stable bonds that prevent leaching of the enzyme, as well as easy recovery and reuse. [3]

Materials & Methods

- Immobilization screening with different covalently linked carriers (Table 1)

Table 1: Carriers used for immobilization (Purolite® Lifetech™ ECR Enzyme Immobilization Resins [4])

Carrier/ Order number	Immobilization type	Pore diameter/ Å	Particle diameter/ µm	Hydro-phobicity
Amino C2 methacrylate/ ECR8309M	Covalent via pre-activation with glutaraldehyde	600-1200	300-710	Hydrophilic
Amino C6 methacrylate/ ECR8409M	Covalent via pre-activation with glutaraldehyde	600-1200	300-710	Hydrophilic
Epoxy methacrylate/ ECR8209M	Covalent	600-1200	300-710	Hydrophilic
Epoxy methacrylate/ ECR8204M	Covalent	300-600	300-710	Hydrophilic
Epoxy/butyl-methacrylate/ ECR8285	Covalent	450-650	250-1000	Hydrophobic

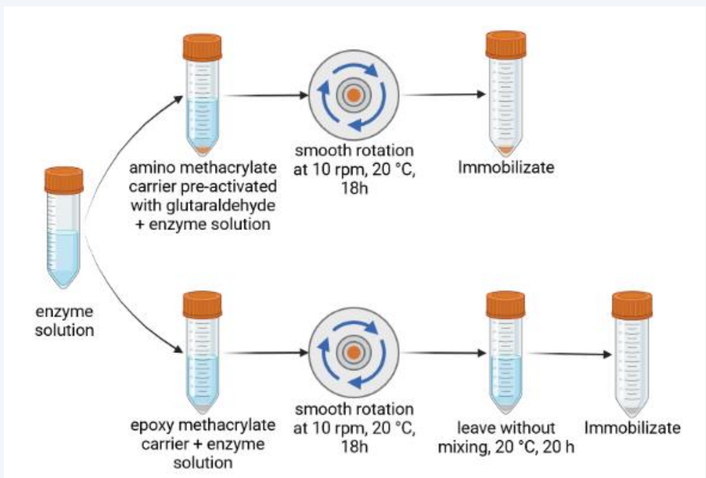

Fig. 3: Immobilization protocol (created in BioRender.com)

- Immobilization was carried out according to the manufacturer's instructions (Fig. 3). [4]
- Immobilization buffer:
  20 mM sodium phosphate buffer pH 7.4 (amino methacrylate),
  1 M sodium phosphate buffer pH 7.4 (epoxy methacrylate)

Experimental Results

- The highest activity and activity yield was achieved with the epoxy carrier ECR8209M (Fig. 4).
- The differences in activity and activity yield (Fig. 4) may be due to the method of binding to the aldehyde group (pre-activated amino methacrylate carrier) via an amino group, or for the epoxy group via thiol, carboxyl and phenol groups. [4]
- The Immobilizate was stable during storage over a period of 2 weeks (Fig. 5A) and reusable (Fig. 5B).

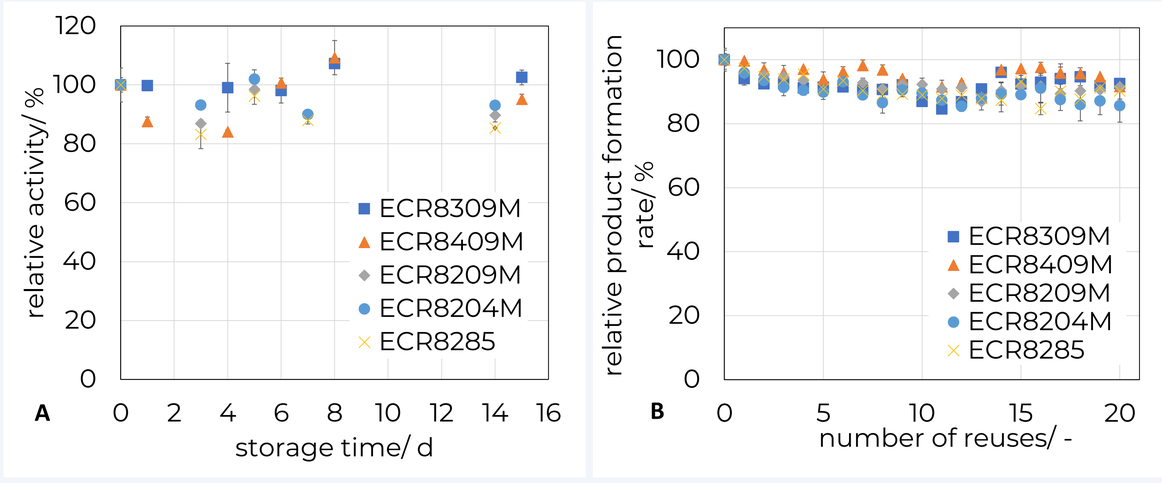

Fig. 5: Stability analysis of RpPPK2-3 immobilized on different carriers, A: Storage stability study, B: Reusability study (Storage conditions for A: T= 6°C, 20 mmol·l^{-1} sodium phosphate buffer pH 7.4/ Reaction conditions activity assay for A and B: 7.3 g·l^{-1} immobilizate, T= 20 °C, 1000 rpm, 50 mmol·l^{-1} Tris buffer pH 7.8, 30 mmol·l^{-1} $MgCl_2$, 7,3 g·l^{-1} PolyP, 5 mmol·l^{-1} CDP, V= 1.5 ml / B: Reaction time per cycle=15 min; after each measurement, the immobilizate was washed twice with 1 ml 20 mmol·l^{-1} sodium phosphate buffer pH 7.4) Error bars show standard deviations of two independent experiments.

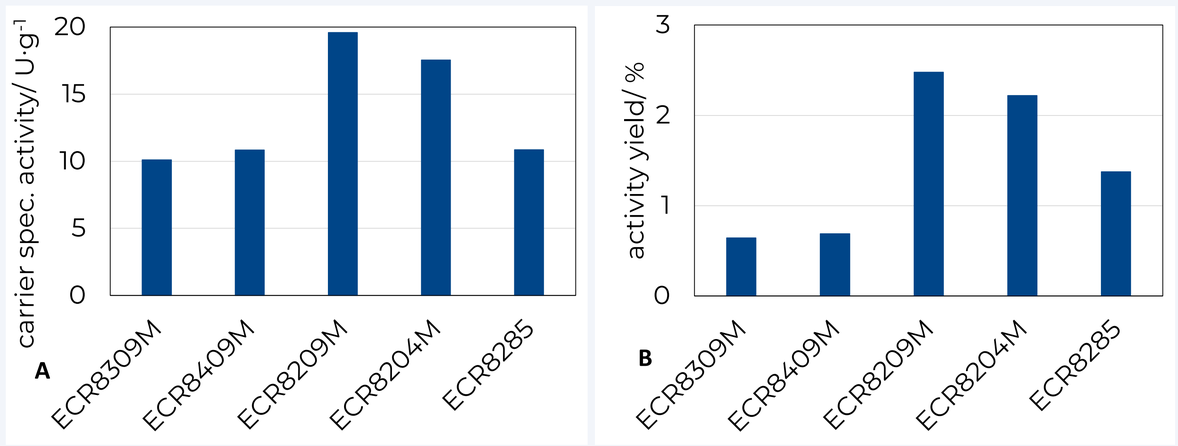

Fig. 4: Carrier spec. activity (A) and activity yield (B) of the with different carriers immobilised RpPPK2-3 (1 Unit (U) is defined as a product formation of 1 µmol per min; Reaction conditions activity assay: 7 g·l^{-1} immobilzate, T= 20 °C, 1000 rpm, 50 mmol·l^{-1} Tris buffer, pH 7.8, 30 mmol·l^{-1} $MgCl_2$, 7.3 g·l^{-1} PolyP, 5 mmol·l^{-1} CDP, V= 1.5 ml, reaction time: 2.5 min, CTP was analysed by HPLC; The activity yield describes the percentage of the apparent activity from the theoretical maximum activity)

Conclusion & Outlook

- Highest activities of the immobilizate was achieved with ECR8209M.
- High stability with all evaluated covalent linking carrier materials was proven in a storage stability and reusability study.
- Further experiments will be performed with ECR8209M.
- High stability and reusability is advantageous for prolonged use in a continuous packed bed reactor.

References: [1] https://www.uniprot.org/uniprot/Q5LSN8 (accessed Mar. 09, 2021); [2] Nahalka, J. et al., Organic and Biomolecular Chemistry 2009, 7(9), 1778-1780; [3] Bolivar, J.M. et al., Chemical Society Reviews 2022, 51, 6251-6290; [4] Purolite Life Sciences, ‚Enzyme Immobilization resins', 2021

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