

Boosting the Kinetic Efficiency of Formate Dehydrogenase

Kinetic Efficiency is Increased by 300% by Combined Effects of High Pressure and Co-solvent Mixtures

Nicolás Gajardo-Parra, Gabriele Sadowski, Christoph Held

The efficiency of homogenous liquid biocatalytic reactions depends on the environment of the catalyst (here: an enzyme) in the solution. This work evaluated the combined effects of temperature, pressure, and co-solvents to increase the kinetic efficiency of NADH synthesis. NADH is a high-value compound (> 10€/g NADH). The synthesis uses formate as substrate, which is oxidized in an aqueous solution with the enzyme formate dehydrogenase. The kinetic efficiency could be increased by 300% applying a pressure of 2kbar and a mixture of the co-solvents dextran and trimethylamine N-oxide (TMAO), compared to an aqueous buffer solution at 1bar. The thermodynamic framework based on the equation of state ePC-SAFT allowed correctly predicting the combined effects of high pressure and co-solvent mixture on the kinetic efficiency without fitting any model parameters to the experimental kinetic data of the co-solvent mixtures.

The application of co-solvents and high pressure is an efficient means to modify the kinetics of enzyme-catalyzed reactions in aqueous solutions without decreasing enzyme stability. This is usually not possible by temperature treatment. FDH (formate dehydrogenase) catalyzes the formate oxidation to CO₂ via a complex mechanism partially limited by an irreversible hydride transfer. From a process perspective, it is most important to tune the macroscopic kinetic parameters at steady state. Thus, the kinetic parameters catalytic constant (k_{cat}), Michaelis constant (K_M), and catalytic efficiency ($K_{eff} = k_{cat}/K_M$) were studied in this work. It was known from literature (cf. publication) that K_{eff} was increased by a factor of three upon raising temperature from 25 °C to 45 °C. We were then keen on studying further the benefit of high pressure and cosolvent addition. TMAO and dextran were used as they improve the thermal stability of FDH. The combined effects of pressure and co-solvent on K_{eff} are shown in Figure 1.

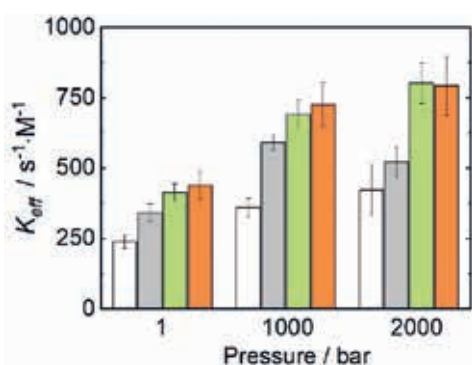


Figure 1: Experimental pressure dependence of kinetic efficiency K_{eff} for the FDH-catalyzed reaction at 25 °C and pH = 7.5 in aqueous buffer solution (white bars) and in aqueous solutions containing co-solvent dextran (gray, 13 mmol/kg), TMAO (green, 1 mol/kg), and a mixture of both co-solvents (orange).

Ultimately, the combined effect of co-solvent addition and high pressure increases K_{eff} by 300% compared to a buffer solution at 1 bar. This boost is mainly caused by favored interactions of formate and TMAO and by mitigating confor-

mational changes in the active pocket of FDH at high pressures. Conversely, dextran causes favorable FDH-solvent interactions to increase k_{cat} . To conclude, the mixture of the co-solvents positively affects both, kinetics and stability of FDH. However, there is a huge matrix to study the kind and concentration of co-solvent candidates. Thus, a thermodynamic activity-based framework was applied to predict the impact of cosolvent mixtures on the kinetic parameters beforehand. ePCSAFT was applied to predict interactions between formate/solvent and FDH/solvent as a function of pressure and co-solvent mixture. As shown in Figure 2, the ePC-SAFT predicted K_{eff} values are in accurate agreement with the experimental data. This is an excellent result as no ePC-SAFT parameters were fitted to the experimental kinetic data of the co-solvent mixtures. The predictions require only K_{eff} data in buffer as input data.

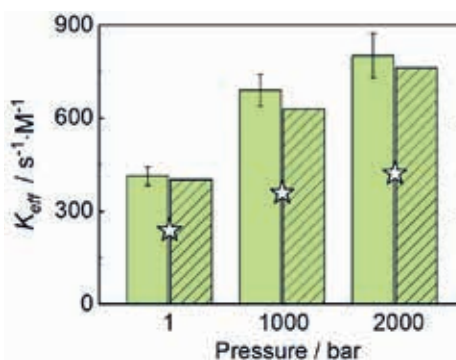


Figure 2: ePC-SAFT modeling of kinetic efficiency. Conditions: 25 °C, pH = 7.5 and 1 mol/kg of TMAO in water. Experimental data (green), ePC-SAFT (green striped), and the TMAO-free values (white stars).

Thus, combining experimental data and thermodynamic predictions allows boosting the kinetic parameters of enzyme-catalyzed reactions. This approach will further contribute to a broader insight into enzyme-catalyzed reactions at crowded cellular conditions, which still remain largely unexplored.

Contacts:

nicolas.gajardo@tu-dortmund.de
christoph.held@tu-dortmund.de
gabriele.sadowski@tu-dortmund.de

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