**A Thermodynamic Approach to Predict the Combined Influence of High-Pressure and Co-Solvents on Reaction Kinetics of a Peptide Hydrolysis.**

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**Highlights**

* Application of a thermodynamic activity-based approach to enzyme kinetics.
* Prediction of combined high-pressure and co-solvent effects on reaction kinetics.
* Molecular interactions explain observed high-pressure and co-solvent effects.

**1. Introduction**

To optimize biotechnological production processes, knowledge about the effects of the reaction medium (temperature, pH, concentration and co-solvents [1]) on reaction yield and kinetics is very important. Even though liquid-phases are generally assumed to be incompressible, pressure is also an important influence factor to tune enzyme-catalyzed reactions taking part in liquid aque­ous systems [2]. Certain enzymes are known to be pressure tolerant and additionally piezophile (i.e. pressure has a positive effect on enzyme activity) [3]. Consequently, the thermo­dynamic vari­able pressure should be an important influence factor, similar to temperature. In this work, the enzyme-catalyzed peptide hydrolysis of SPNA (N-succinyl-L-phenylalanine-p-nitroanilide) was in­vestigated. The effects of high pressure and of co-solvents on the reaction kinetics were studied and explained by thermodynamics (interactions in the liquid phase).

**2. Methods**

In this work, experimental kinetic studies were performed at 20 °C for pressures of 1 bar and 1500 bar and the high-pressure influence on reaction kinetics was determined. Experimental data was analyzed according to the Michaelis-Menten procedure yielding the observed Michaelis constant and the turnover number . Further, the thermodynamic model PC-SAFT (Perturbed-Chain Statistical As­sociating Fluid Theory) [4] was used to predict co-solvent effects on the reactive system. For this purpose, the constants and were determined based on thermodynamic activities instead of concentrations in order to be independent of solvent effects, which requires activity coefficients: .

The substrate’s activity coefficient as well as the enzyme’s activity coefficient were obtained by PC-SAFT. These account for molecular interactions that are expected to determine co-solvent effects on reaction kinetics. The kinetic constants were measured only in the neat cosolvent-free system while PC-SAFT was then applied to pre­dict the kinetic constants in a reactive system of different composition or in different solvents. Applying the exponential pressure dependence of and additionally allowed for predicting the pressure influence on reaction kinetics.

**3. Results and discussion**

Experimental results showed the positive influence of high pressure on the reaction kinetics. For increasing pressures up to 2000 bar, was found to decrease indicating a higher affinity of the substrate towards the enzyme. Furthermore, increased for increasing pressure indicating a faster product formation. In contrast, the co-solvents under investigation (0.5 mol kg-1 TMAO, 1 mol kg-1 urea and 4.2 mol kg-1 DMSO) had a negative effect on reaction kinetics (see Fig­ure 1). These effects were rather small for TMAO and urea by only slightly increasing and de­creasing . However, DMSO strongly increased , indicating a weaker affinity of the substrate towards the enzyme. was also considerably decreased by DMSO.



**Figure 1.** Left: Experimentally determined *KM* (black) and PC-SAFT predicted *KM* (grey) in mmol kg-1, right: Experimentally determined *kcat* (black) and PC-SAFT predicted *kcat* (grey) in s-1 plotted against co-solvent at 500 bar and 20 °C.

PC-SAFT predictions of high-pressure and co-solvent effects on reaction kinetics were per­formed. For these predictions, the combined high-pressure and co-solvent effects on reaction kinetics were of special interest (see Figure 1). The PC-SAFT predicted Michaelis constants are in almost quantitative agreement with experimental data for all co-solvents as well as for all ob­served pressures. The fact that PC-SAFT successfully predicts the combined co-solvent and high-pressure effects on is a proof that the observed effects are dominated by molecular interac­tions. Additionally, even though the combined co-solvent and pressure effects on are small, PC-SAFT predictions agree very well with experimental data.

**4. Conclusions**

Applying a thermodynamic activity-based approach allows predicting the combined high-pressure and co-solvent effects on reaction kinetics of the investigated peptide hydrolysis. Predictions and ex­perimental results were in very good agreement. That is, molecular interactions (sub­strate/ co‑solvent) are mainly responsible for the experimentally-observed effects of high pressure and co‑solvent on reaction kinetics.

**References**

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