**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 $K\_{M}^{obs}$ and the turnover number $k\_{cat}^{obs}$. 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 $K\_{M}^{a}$ and $k\_{cat}^{a}$ were determined based on thermodynamic activities instead of concentrations in order to be independent of solvent effects, which requires activity coefficients: $K\_{M}^{a}=K\_{M}^{obs}∙γ\_{substrate} and k\_{cat}^{a}=k\_{cat}^{obs}∙γ\_{enzyme}^{\*}$.

The substrate’s activity coefficient $γ\_{substrate}$ as well as the enzyme’s activity coefficient $γ\_{enzyme}^{\*}$ 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 $K\_{M}$ and $k\_{cat}$ 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, $K\_{M}$ was found to decrease indicating a higher affinity of the substrate towards the enzyme. Furthermore, $k\_{cat}$ 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 $K\_{M}$ and de­creasing $k\_{cat}$. However, DMSO strongly increased $K\_{M}$, indicating a weaker affinity of the substrate towards the enzyme. $k\_{cat}$ 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 $K\_{M}$ 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 $K\_{M}$ is a proof that the observed effects are dominated by molecular interac­tions. Additionally, even though the combined co-solvent and pressure effects on $k\_{cat}$ 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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