An alternative kinetic evaluation of a reversible enzyme reaction

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One of the most investigated biochemical pathways is the glycolysis. While searching in literature databases, more than 35.000 publications can be found. Some of these publications studied the thermodynamics of the glycolysis and have postulated that this pathway is not thermodynamically feasible [1-4]. A distributed bottleneck was identified between reaction 5 and 6 but there are also some local bottlenecks with a single unfeasible or undetermined reaction [2, 3]. One example for such a reaction is reaction 9 of the glycolysis where the conversion from 2-phosphoglycerate to phosphoenolpyruvate takes place which is not irreversible but an equilibrium reaction. Thermodynamic and kinetic investigations of this reaction were done previously but just with standard instead of cell mimicking conditions. In addition to taking these conditions into account new thermodynamic research of this reaction should also include a kinetic analysis meaning that an equilibrium reaction cannot be interpreted using the usual Michaelis-Menten kinetics. The backward reaction from the product to enzyme-substrate-complex is neglected by this classical approach. Strangely enough, the most kinetic studies on reaction 9 of the glycolysis evaluate the data using the Michaelis-Menten model and therefore disregard the reversibility of the reaction [5-9]. There are some models which incorporate the reaction equilibrium, like the Hoh and Cord-Ruwisch approach [10] or the reversible Michaelis-Menten mechanism [11-17]. The reversible Michaelis-Menten mechanism results in a bad parameter fit due to of the high number of adjustable parameters [15, 18]. Therefore, we want to present an alternative way for data analysis of reversible reactions that combines a kinetic approach with irreversible thermodynamics and drastically reduces the number of required parameters. In addition to this fundamental result, we were able to describe quantitatively the influences of the different cell mimicking conditions on the kinetics of the reaction. The imitation of the crowding inside cells decreases the kinetics the most. The temperature dependency of our new kinetic constants follow the well-known Arrhenius relation. The dependency on the pH was also strong. Magnesium and ionic strength showed a weaker dependency. In the further course of the study, we want to apply our model to other reversible reactions of glycolysis and explore the predictive potential of thermodynamics for systems biology.

Keywords isothermal titration calorimetry; irreversible thermodynamics; glycolysis; reversible reaction

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