**Feasibility Study of Glycolysis Using New Thermodynamic Standard Data.**

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**Highlights**

* Thermodynamic approach to describe reaction equilibria.
* New consistent standard Gibbs energy of reaction for glycolytic reactions.
* Thermodynamic explanation of the feasibility of the glycolysis pathway.

**1. Introduction**

Thermodynamics has successfully been applied to many areas of biological systems. However, application of thermodynamics to examine entire metabolic networks remains a challenging task. A prominent example for such a network is the enzymatic reaction cascade of the glycolysis pathway. The feasibility of a pathway can be explained by means of second law of thermodynamics, i.e. by the rule that Gibbs energy of reaction ΔRg has to be negative for each single reaction. Applying this to glycolysis leads to positive ΔRg values for some reactions, i.e. thermodynamic analyses using literature standard data predict that glycolysis is not feasible [1]. We found [2] that reasons for this phenomenon are inconsistent published data on reaction equilibria of the single reaction steps.

**2. Methods**

The Gibbs energy of reaction ΔRg is determined using standard Gibbs energy of reaction ΔRg0 and the cellular metabolite activities (expressed as ratio of products and reactants Q) and solving ΔRg = ΔRg0 + RTlnQ. In recent works it was found that published ΔRg0 values were not determined under standard conditions (hypothetically ideal solution) [2,3]. This causes inconsistent values for ΔRg0 and thus also unreliable ΔRg values. ΔRg might even be positive though the reaction has been found to be feasible under the given conditions, which leads to wrong conclusions when examining metabolic networks. Thus, the main goal of this work was to understand the thermodynamics of each single reaction step within glycolysis. Therefore, reaction equilibria were measured in-vitro and influences of the reaction medium on reaction equilibria were determined experimentally. In order to bring these experimental conditions to standard state, activity coefficients of the metabolites were measured and modeled with the electrolyte Perturbed-Chain Statistical Associating Fluid Theory (ePC-SAFT) [4].

**3. Results and discussion**

Combination of experimental equilibrium concentrations and activity coefficients allowed establishing an activity-based approach to describe reaction equilibria while simultaneously providing consistent standard data ΔRg0 for the single reactions of glycolysis. Based on the new ΔRg0 values and the activity coefficients of the metabolites, the feasibility of glycolysis could be proven in this work. This is shown in Figure 1, in which ΔRg values calculated from literature and from new ΔRg0 values are compared. The comparison shows, that there is a significant difference between the literature and the new data. Using new data from the introduced approach does even result in negative ΔRg values, where using literature data resulted in positive values, which shows the relevance of new high precision standard data. Additionally, applying the new ΔRg0 values and the activity-based approach allows predicting the influence of the reaction medium (concentrations of the metabolites, salts, pH value, temperature and cosolutes) on reaction equilibria.

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**Figure 1.** Gibbs energy of reaction calculated from literature (black) and new (grey) standard Gibbs energy of reaction for the ten single reaction steps of glycolysis pathway.

**4. Conclusions**

Inconsistent published data on reaction equilibria and standard Gibbs energy of reaction ΔRg0 have been identified to be the reason why thermodynamic analyses failed to explain the feasibility of glycolysis. Thus, an activity-based approach has been developed which uses concentration-based equilibrium measurements and activity coefficients from modeling with ePC-SAFT in order to provide new consistent ΔRg0 values. Based on this approach, the feasibility of glycolysis could be proven in this work. Additionally, applying the new ΔRg0 values and the activity-based approach allows predicting the influence of the reaction medium on reaction equilibria.

**References**

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