Theses

of the PhD Thesis

Internal friction in enzyme reactions

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Introduction: background and aims

In order to characterize and approach experimentally the general features of enzyme reactions, empirical parameters are usually introduced, which simplify the complex energy landscape of enzyme reactions. The long-known parameters from Arrhenius plots and Eyring-Polányi plots such as activation energy, preexponential factor and activation entropy describe the observed temperature dependence of enzyme reactions. For reactions in condensed phase Kramers introduced a friction parameter that describes the effects of the constant energy exchange between the reactants and their surroundings (1, 2). Kramers' formula was adapted to enzyme reactions in a way that the friction was attributed to two sources: external friction due to the movement of solvent molecules and internal friction due to the reorganization of the protein interior (3). The sum of these two types of friction was inserted into the Kramers' formula while other parameters (activation energy, ΔE_a and a temperature and viscosity independent preexponential factor, A) characterizing the Arrhenius-like temperature (T) dependence were kept:

$$k(T,\eta) = \frac{A}{\sigma + \eta} \exp\left(\frac{-\Delta E_a}{k_B T}\right) \qquad \text{Eq 1}$$

As friction originating from the solvent is directly proportional to its viscosity (η), in the equation, the friction inside the protein is also

characterized by a parameter (σ) with units of viscosity to match dimensions. This newly introduced σ parameter is referred to as internal viscosity, which is in the focus of this study.

The model enzyme – human trypsin 4 (HuTry4)

Trypsin in the body is expressed as trypsinogen, its inactive, zymogen form. Upon a proteolytic cleavage a conformational change occurs, which transforms trypsinogen from an inactive zymogen conformation to its active form (Figure 1 A). The zymogen and the active forms are stable conformations of trypsinogen and trypsin, respectively, and their atomic crystal structures are available (1TGN, 2PTN)(4, 5). In vitro a similar, single-step activation reaction can be initiated in trypsin by a rapid jump from pH 11 to pH 8 and the reaction can be followed by the change of the intrinsic Trp fluorescence in a stopped-flow apparatus (6, 7), which provides an excellent experimental system for the investigation of the conformational change during activation.

It was found that a domain rearrangement occurs during trypsinogen activation, i.e. four loops of the activation domain rearrange by rotating around five glycine hinges (residues 19, 142, 184, 193, 216, Figure 1 B) (5, 6). Except for Gly193, the Ala mutations of these glycines dramatically reduce the proteolytic activity of trypsin (8). Recently it was shown that activation slowed by replacing Gly with Ala or bulkier sidechains (7) at position 193. While the slope of the Arrhenius plots remained unchanged (Figure 1 C), the viscosity

dependence of the reactions changed drastically (Figure 1 D), which implied that the mutations at this hinge position (Gly193) cause highly specific changes in the apparent internal viscosity parameter (σ) of activation without affecting the structural and activity properties of the protein.

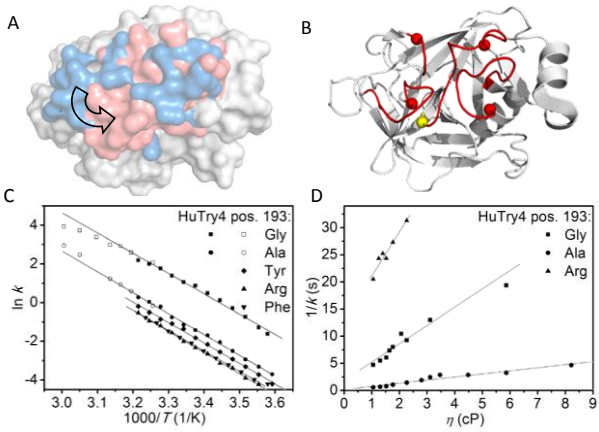

Figure 1 Trypsin activation: HuTry4

A) The overlaid atomic structures of bovine trypsin in active (2PTN) and zymogen (1TGN) conformation. During activation the activation domain moves from the zymogen (blue) to active (red) conformation. B) The active structure of bovine trypsin (2PTN). The four loops of the activation domain are shown in red (residues 16-19, 142-152, 184-194, 216-223). The Gly residues bordering these loops are shown as spheres, position 193 is in yellow. These Gly residues have been suggested to work as hinges during loop rearrangement (5). C) Arrhenius plots of HuTry4 activation for the different mutants at position 193. D) Viscosity dependence of the activation of HuTry4. Data for C and D are taken from (7).

Trypsin activation is thus an ideal model process for the investigation of the internal friction during enzymatic conformational changes, because it is a well defined single-step reaction between two conformers manifested by the rotation of a distinct domain around a

few glycine hinges, and mutations at the hinge position 193 specifically affect internal viscosity. 193A and 193G mutants of human trypsin 4 (HuTry4) were chosen as model enzymes for this study.

We aimed to characterize the physical and structural background of the internal viscosity phenomenon. The following scientific questions were in the focus: i) The physical background of internal friction was approached by investigating its temperature dependence. Specifically, the temperature and viscosity dependence of the rate constant of HuTry4 activation were determined. ii) The relations of internal friction to the structural characteristics of the protein were approached by investigating a Gly-Ala exchange at an important hinge position. Specifically we determined how the exchange of Gly to Ala at position 193 in HuTry4 affects the internal viscosity during trypsinogen activation. iii) Finally, we aimed to construct a comprehensive model that explains our observations and relates internal viscosity to the well established parameters describing the energy landscape.

Methods

Transient kinetic methods were used to characterize the rate of conformational change during the activation of trypsin: upon rapid mixing with a stopped-flow apparatus a pH jump triggered the activation of the 193A or 193G HuTry4 enzyme in solutions of different viscosity and temperature. This activation resulted in a measurable Trp

fluorescence intensity change that was followed in time (Figure 2). Maltose was chosen as cosolvent for controlling viscosity, as it is relatively small, and the ratio of viscosity change and dielectric constant change is the greatest among simple saccharides. Maltose concentration in the solutions varied from 0 to 1 M with 0.1 M increments resulting in viscosities between 0.6 cP and 6 cP.

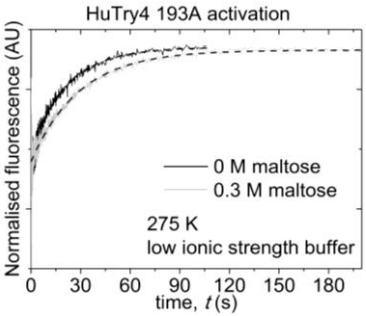

Figure 2 Typical transient kinetic fluorescence traces.

Representative single fluorescence transients illustrate the slowing effect of viscosity on trypsin activation. The fluorescence change upon the activation of HuTry4 193A is shown with (grey) and without (black) viscogen. The dashed lines are the fitted exponential decay curves.

The measured rate constants were analyzed as a function of both viscosity and temperature (Figure 3). The viscosity dependence confirmed the validity of Eq 1 (Figure 3 B), and internal viscosity values were calculated at every temperature for both HuTry4 193G and 193A. The temperature dependence of the rate constants produced classical Arrhenius plots (Figure 3 C) and was consistent with previous publications. Bivariate analysis of the data gave accurate fitting results (Figure 3 A).

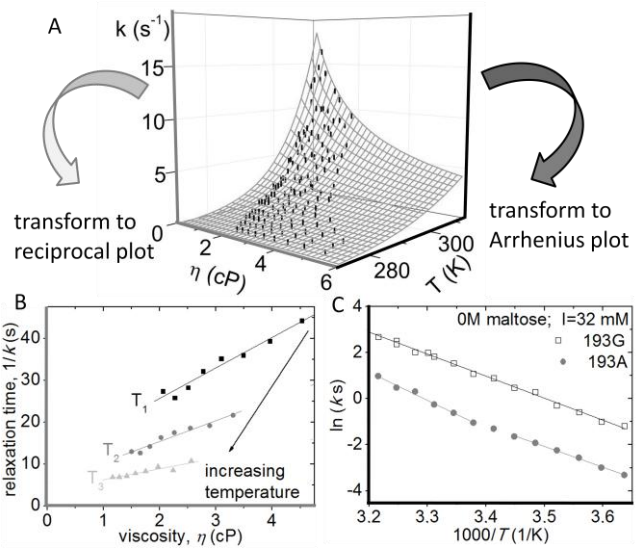

Figure 3 Temperature and viscosity dependence of trypsin activation

A) The measured rate constants for 193G HuTry4 are plotted against both viscosity and temperature. The fitted surface is based on Eq 1 and Eq 2 (see Results). B) Viscosity-dependence of the relaxation time. Data are representative measurements with 193A HuTry4 in at 275, 283 and 287K, ionic strength I=32 mM. C) The temperature-dependence of rate constants in buffer without viscogen.

Results

Thesis 1 Internal viscosity has Arrhenius-like temperature dependence, it depends exponentially on the reciprocal of temperature:

$$\sigma(T) = \sigma_0 \exp\left(\frac{\Delta E_\sigma}{k_B T}\right) \qquad \text{Eq 2}$$

where the characteristic energy ΔE_σ is the activation energy of internal friction and σ_0 is a temperature independent prefactor.

Thesis 2 In HuTry4, at position 193 the Gly-Ala switch modifies the internal viscosity parameter, especially the temperature dependence of internal viscosity. The 193G HuTry4 activation has higher internal viscosity than 193A HuTry4 activation.

Thesis 3 The change in ionic strength does not affect the temperature dependence, but changes the viscosity dependence of HuTry4 activation in a way that higher ionic strength results in stronger viscosity dependence.

Thesis 4 We constructed a coherent energy landscape model for HuTry4 activation, which attributes the roughness of the energy landscape to internal friction. (Figure 4)

Thesis 5 From systematic temperature and viscosity dependence data iso-viscous Arrhenius plots were constructed for HuTry4 activation, which are not biased by the temperature-dependent change in viscosity of the solvent.

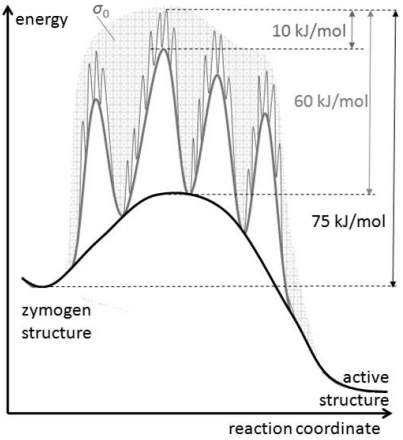

Figure 4 Energy landscape of HuTry4 activation.

The reaction coordinate represents the transition of HuTry4 193A/G from zymogen to active structure. Two levels of roughness are represented, which – depending on flexibility – correspond to the activation energy of internal viscosity in Eq 2. Shading illustrates friction along the reaction coordinate.

Conclusions

This work was centered around three specific questions, i) What can we learn about the physical background of the empirically introduced internal friction parameter by measuring its

temperature dependence? ii) What can we learn about the relationship of internal friction and the structural properties of enzymes? iii) How can we model the observed viscosity and temperature dependence of reactions, how can we relate internal viscosity and the well known parameters of the energy landscape?

We found that internal viscosity has an Arrhenius like temperature dependence, which suggests that similarly to the viscosity of liquids or other friction it is related to barrier crossing events. By re-analyzing earlier literature on viscosity and temperature dependence, we found that our observations about the temperature dependence of internal viscosity are also true for other reactions on very different timescales. Based on the observation that the more flexible enzyme had higher internal viscosity while the overall energy barrier calculated from the Arrhenius plot remained unchanged, we constructed a model with elastically coupled reaction coordinates that combines and explains the relationship between the hierarchical roughness of the energy landscape, flexibility and internal friction of enzymes. Moreover, the model resolved two apparent contradictions: i) We distinguished flexibility as a greater amplitude movement from flexibility as elasticity and explained why they react to viscosity in opposite ways. ii) Our model explains why in case of identical energy barriers the more flexible enzyme with greater internal viscosity is faster.

Related publications

Rauscher AA, Simon Z, Szollosi GJ, Graf L, Derenyi I, Malnasi-Csizmadia A

Temperature dependence of internal friction in enzyme reactions

FASEB J 25: (8)2804-2813 (2011)

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IUBMB LIFE 65: (1)35-42 (2013)

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