Critical Review

Internal Friction in Enzyme Reactions

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Abstract

The empirical concept of internal friction was introduced 20 years ago. This review summarizes the results of experimental and theoretical studies that help to uncover the nature of internal friction. After the history of the concept, we describe the experimental challenges in measuring and interpreting internal friction based on the viscosity dependence of enzyme reactions. We also present speculations about the structural background of this viscosity dependence. Finally, some models about the relationship between the energy landscape and internal friction are outlined. Alternative concepts regarding the viscosity dependence of enzyme reactions are also discussed. ©2012 IUBMB Life, 65(1):35–42, 2013

Keywords: *enzyme mechanisms; viscosity dependence; internal friction; energy landscape; roughness; Arrhenius plot; Kramers theory*

You cannot step twice into the same stream.

Heraclitus

Introduction

If we consider the conformational diversity and dynamics of proteins, we may end up with the same conclusion as Heraclitus since "it is unlikely (…) that a protein returns twice to the same [conformational] substrate" [cited from (1)] The enormous conformational space of a protein can be characterized theoretically with an energy landscape by assigning an energy/probability to each conformational state [for an insightful review on the energy landscape and the dynamics of proteins see (2)]. By the knowledge of the complete energy landscape, the conformational transitions, enzyme reactions, folding–unfolding processes, and so forth could be precisely predicted. However, the detailed energy landscape cannot be determined experimentally because of its vast complexity. Thus, empirical parameters are usually introduced to characterize the general features of enzyme reactions. Activation energy, pre-exponential factor, and activation entropy are long-known parameters that describe the observed temperature dependence of enzyme reactions. Another more recently introduced parameter is the internal viscosity, which characterizes the viscosity dependence of reactions.

For a comprehensive and practically useful picture, the infinitely complex energy landscape and the empirical parameters should converge: the former has to be simplified, whereas the latter should be refined in more detail. Ideally, the empirically introduced parameters will become physically meaningful, by reflecting the important characteristics and structure of the energy landscape. This could provide a physically justified, simplified energy landscape model that could be approached experimentally and predict protein dynamics. The present review focuses on internal friction in single, first-order reaction steps of enzyme reactions, whereas folding processes have been reviewed recently (3).

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Experimental Determination of the Main Characteristics of the Complex Energy Landscape

The first experimental approach toward obtaining a simple energy landscape—the activation energy barrier—for chemical reactions was the determination of the temperature

TABLE 1 *Range of published internal viscosity values in enzyme reactions*

Range of internal viscosity values (cP)	*Types of enzymes or reaction studied*
2–7.46	Myoglobin (7,10,11,12,13)
0.2–28	Trypsin (14,15)
0.8–30[a]	Electron transfer (9,16,17,18)
1.5–5	Horseradish peroxidase (19)
0.18[a]	Proton tunneling (20)

[a] *Values recalculated based on Eq. (2).*

dependence of the rate constant, producing the Arrhenius plot. At the molecular level, the theoretical background of the Arrhenius equation was provided by the transition state theory, which is a good approximation for gas phase reactions of small molecules. Later this theory was extended by Kramers for first-order reactions in condensed phase by introducing parameters that describe the effects of the constant energy exchange between the reactants and their surroundings (4,5). In the highly damped regime, which is supposedly the case in enzyme reactions, the Kramers formula predicts that the rate constant (k) is inversely proportional to the friction coefficient. Based on the Stokes law, this friction coefficient is usually considered proportional to the viscosity of the solvent (η):

$$k(T,\eta) = \frac{A}{\eta}\exp\left(\frac{-\Delta E_a}{k_B T}\right) \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, ΔE_a is the activation free energy, and A is a temperature and viscosity-independent constant. As enzyme reactions are accomplished in condensed phase and the reactants are large, complex molecules, the approximation of the energy landscape by Kramers theory came into the focus of interest. Nevertheless, several early experiments indicated that Kramers theory was not sufficient to describe the viscosity dependence of certain enzyme reactions because weaker than linearly proportional viscosity dependences of the relaxation time of the reaction (the reciprocal for the rate constant) were found (6,7).

Linear Viscosity Dependence of Enzyme Reactions: The Concept of Internal Friction

To explain the observed deviation from Kramers theory, the concept of internal friction was introduced. During a conformational change, friction characterizes the energy and momentum exchange caused by the movement and collision of all atoms that are involved in the reaction (8). For an enzyme reaction, the friction encountered by the protein comes from two sources: friction due to the movement of solvent molecules (external friction) and friction due to the reorganization of the protein interior (internal friction) (7). The sum of these two types of friction was inserted into the Kramers relationship:

$$k(T,\eta) = \frac{A}{\sigma+\eta}\exp\left(\frac{-\Delta E_a}{k_B T}\right) \quad (2)$$

As friction originating from the solvent is directly proportional to its viscosity (η), the friction inside the protein is also characterized by a parameter (σ) with units of viscosity to match dimensions, and is often referred to as internal viscosity.

We note that without much justification another, admittedly the empirical mathematical formulation of internal viscosity appeared in the literature as the $1/(\sigma + \eta)$ term of Eq. (12) was changed to $(\eta_0 + \sigma)/(\eta + \sigma)$, where $\eta_0 = 1$ cP (9). This modification predicts a rather unlikely behavior: when $\eta > \eta_0$, the rate constant increases with increasing internal viscosity, rendering this formula less appealing.

Experimental Values of Internal Viscosity

Based on Eq. (2), the internal viscosity of several enzyme reactions was published in the last 20 years. In most of the studies, the experimentally determined values of the internal viscosity are in the range of the viscosity of aqueous solvents (Table 1). In the Supporting Information Table S1, we also list the internal viscosities that we calculated from published viscosity dependence data of different enzyme reactions. We have to note here that not all viscosity-dependence data fit Eq. (2). There exists an alternative fitting formula, which is discussed later in detail. However, in most cases, Eq. (2) or its alternative fit equally satisfactorily because of the applied narrow viscosity range. Despite the fact that σ has a viscosity dimension, it should not be interpreted as the viscosity of some liquid. Rather, it is more meaningful in a relative sense. Its value relative to the solvent viscosity shows the relative importance of energy and momentum exchange within the protein and between protein and solvent atoms. The same protein, as exemplified by several experiments, can even exhibit very different internal viscosities in different reactions. Viscosity dependence can be substrate specific (21,22), hydride and proton tunneling can behave differently (20), the different steps of a reaction can have different viscosity dependences (23), all of which demonstrate that internal viscosity is related to a specific reaction or a specific conformational change and it is not an inherent property of the protein itself.

Besides the data that are cited in Table 1, there are a significant number of measurements where no viscosity dependence of the reaction was observed, which was interpreted in

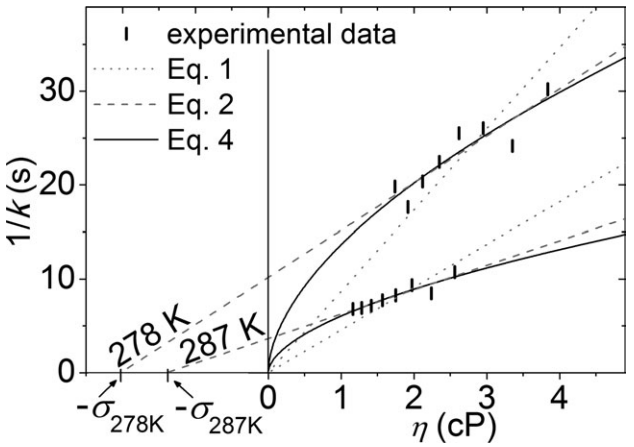

FIG 1

The viscosity dependence of the rate constants. The relaxation time (reciprocal of the rate constant) is plotted against the viscosity at two different temperatures [selected data on trypsin activation (14)]. Because of the restricted viscosity range and the lack of data near zero viscosity, both Eqs. (4) and (2) fit the data well; therefore, no strong conclusion can be drawn as to whether a linear or a power-law relationship describes the viscosity dependence more properly. The linearly proportional dependence—Kramers theory, Eq. (1)—is also drawn for comparison. The horizontal intercept of the linear dependence [Eq. (2)] gives the internal viscosity value (σ) for a given temperature.

different ways: (1) internal friction is very high (20); therefore, the reaction is insensitive to viscosity; (2) no significant conformational change takes place in the reaction or the conformational change is not rate limiting (24); (3) the cosolvent used increases the bulk viscosity of the solution, but the microviscosity is not affected (25). We note here that conclusions about one enzyme may not be easy to transfer to other enzymes; therefore, any of the listed explanations could hold depending on the experimental system. The other extremity of experimental results is when negative internal viscosity values were found, which are difficult to interpret (16) [see also Table S1 in the Supporting Information (20,26)]. These values might come from viscosity independent effects of the applied cosolvents and draw our attention to the limits of the measurements.

Experimental Approaches for Measuring Internal Viscosity of Enzyme Reactions

Internal viscosity is determined indirectly, by measuring the viscosity dependence of the reactions (Figure 1). To vary the solvent viscosity, viscogen cosolvents, for example, glycerol, glucose, sucrose, maltose, polyethylene glycols of different molecular weights, ethanol, methanol, are added to the reaction buffers. This method has some drawbacks that have to be considered in the planning and evaluation of the experiments. Addition of cosolvent changes not only the viscosity but affects also various other features of the solution: dielectric properties, water activity, ionic strength, osmotic pressure are just some of the most important examples. Authors often make great effort to keep the unwanted changes to a minimum (*e.g.*, $<$12% change in the dielectric constant due to cosolvent and $<$ 5% change in ionic strength due to temperature) (14) or prove that the changes are negligible in their specific experimental setup (*e.g.*, changing the pH from 6 to 10 only altered the rate constant of ligand binding to myoglobin by a factor of 2, ionic strength did not have an effect between 1 and 200 mM) (6). Often the effects of different cosolvents are compared within the same setup to check if there are cosolvent-specific effects on the reactions (20,24,27–32). Cosolvents may perturb equilibria especially in folding/unfolding reactions (3) or affect the height of the activation energy barrier (33), but in most reactions of folded enzymes cosolvents are provedor supposed to be (14,34) inert in this sense. In a relatively small concentration range of cosolvents, the unwanted effects might be negligible, but when a large viscosity range is investigated, the perturbation caused by the high concentration of the viscogen is very difficult to interpret. It is especially a serious challenge if one wants to distinguish whether the viscosity dependence of the relaxation time of the reaction is linear or follows a power law, since it requires a large viscosity range (see Figure 1). Moreover, in aqueous solvents, viscosity can hardly be reduced by additives, which causes difficulties in the extrapolation of the rate constant to zero viscosity (see Figure 1).

Computational methods may overcome these technical difficulties. Influencing macroscopic viscosity in simulations was performed by changing the solvent temperature independently of the protein temperature (35), the mass parameter of the solvent molecules (36), or simply using implicit solvent with a damping constant (37–39). These *in silico* experiments are less comprehensive at present, but such approaches could prove useful in complementing *in vitro* studies and theoretical considerations.

Structural Origin of the Viscosity–Sensitivity of Enzyme Reactions

Authors agree that the viscosity-dependent reactions must be coupled to some conformational changes. Often the viscosity dependence is a proof for a conformational change on the reaction (9,16,33). There have been many attempts to find structural explanations to the observed differences in internal viscosities or to reveal the mechanism of how the solvent effects are mediated through the protein interior to the active site. Understanding the structural background of the viscosity dependence of reactions can bring us closer to understanding the structural background of internal friction.

Four main ideas about the structural background of viscosity dependence are illustrated by a simplified model

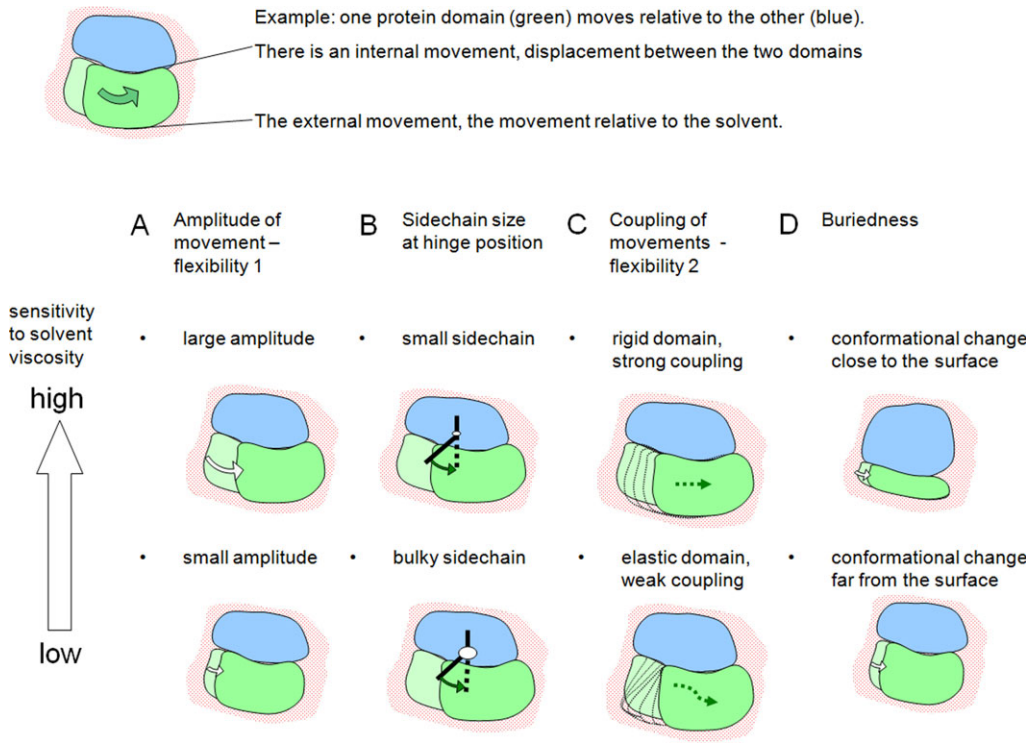

FIG 2

Structural speculations about the viscosity sensitivity of conformational changes. In the model used for illustration, we consider a simple conformational change: sliding of the lower protein domain (green) relative to the static, upper domain (blue). The initial conformation of the sliding domain is shown in lighter color. Four different structural characteristics are evaluated based on their effect on viscosity dependence. (A) The greater the amplitude of the conformational change, the greater the sensitivity to viscosity. (B) When the two domains are connected, the sliding movement causes rotation in the connecting segments. The amino acids with the greatest rotation function as hinges. If these amino acids are bulkier, viscosity dependence decreases. (C) The conformational change takes place along two reaction coordinates, or two interaction surfaces; there is an internal surface, that is, the surface between the two domains and an external surface, which is the surface of the protein moving relative to the solvent. These two surfaces are connected through the sliding (green) domain itself; consequently, the reaction coordinates are coupled. The extent of coupling between the two reaction coordinates depends on the elasticity of the sliding domain. The stronger the coupling, the higher the sensitivity to viscosity. (D) Movements far from the solvent-protein interaction surface are less sensitive to changes in viscosity; therefore, the more buried the conformational change, the smaller the viscosity dependence. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

conformational change of two protein domains moving relative to each other on Figure 2. (i) It was found that viscosity dependence is influenced by the amplitude of the conformational change (29), experiments in sugar glasses also support this finding: even though the viscosity is higher than 10^{15} cP, small-amplitude dynamics remain unchanged while large-amplitude dynamics are dampened (40–42) [(Figure 2(A)]. (ii) Tóth et al. observed (15) that in trypsin activation, the size of the side chain of the residue at the hinge, where the relative rotational movement of the two domains occurs, is a major determinant of the sensitivity to viscosity [Figure 2(B)]. (iii) Steinhoff found (43) that the more buried a region in a protein, the less sensitive it is to the solvent viscosity [Figure 2(D)]. (iv) Flexibility is also often showed to influence viscosity dependence. The Kostic group compared the rate of electron transfer within a rigid (covalently bound) and a more flexible (electrostatically bound) complex of cytochrome c and plastocyanin. The rigid complex was found insensitive to viscosity, whereas the more flexible complex showed viscosity dependence. They proposed that the more rigid a structure, the less sensitive it is to viscosity (17,24). A similar conclusion was drawn from the Dronpa photoswitching reaction (26), where the more flexible mutant was more sensitive to the external viscosity. The opposite conclusion was drawn from experiments of trypsin activation reaction studying trypsin mutants, where the supposedly more flexible mutant containing a glycine hinge was less sensitive to solvent viscosity than the more rigid alanine mutant (14). The contradiction may be rooted in the vague definition of flexibility as it may describe fundamentally different phenomena: for example, amplitude of movement [Figure 2(A)] or coupling of two movements [Figure 2(C)]. If we assume that flexibility characterizes the amplitude of a conformational change, then a greater conformational change belongs to the more flexible enzyme, which may be the reason why it is more sensitive to solvent viscosity [see the findings of (29,40–42) Figure 2(A), flexibility 1]. However, flexibility may also be used to

TABLE 2 *Studies investigating both viscosity and temperature dependence of reactions*

Type of reaction studied	*Range of viscosity studied (cP)*	*Range of temperature studied (K)*
photoinduced electron transfer between Zn cytochrome c and Cu plastocyanin (9,45)	0.8–790	260–308
photoinduced electron transfer between Zn cytochrome c_6 and cytochrome f (18)	0–60	283, 293, 313
electron transfer between cytochrome f and plastocyanin (46)	1–3	293, 300
proton and hydride tunneling in light-activated protochlorophyllide oxidoreductase (20)	1–10	298–323
horseradish peroxidase phosphorescence quenching (19)	0.9–12	278–298
hemoglobin-bound NO spin label (43)	1–10,000	276–315
Trp phosphorescence lifetime of four different proteins (47)	~1–100	253–333
lysosyme Trp isotope exchange (32)	1–6.3	288, 293, 299
cytochrome C folding from a compact configuration (48)	0.7–6	290, 293, 298, 303
trypsinogen activation (14)	0.9–5.5	275–313
O_2 and CO binding to myoglobin and protoheme (6)	~1–100,000	180–340
CO binding to ferrous microperoxidase 11 (49)	~7–700	253–313
CO binding to myoglobin (34)	~1–10^{10}	63–290
carboxypeptidase A hydrolysis rate (44)	~7–90	283–308
H^+ ATPase (28)	1–3	293, 308, 313

characterize the coupling strength between, for example, the displacements of the outer and inner surfaces of a protein domain without any reference to the amplitude of their movements [Figure 2(C), flexibility 2]. A strong or rigid coupling means that the conformational change happens at the same time on each "venue" (*e.g.*, both inside the protein and at the protein–solvent interface), whereas a weak or flexible coupling means that the two movements may be partially sequential because of the elastic property of the hinge and/or the moving domain (14).

The Physical Background of Internal Friction

There are indications that solvent viscosity may influence the energy landscape by modifying the apparent energy barriers (6,44). One effect is that when measuring the temperature dependence of reactions to determine the activation energy, the apparent activation energy we calculate from the slope is 5 kcal/mol higher (44) than the correct value, as the temperature dependence of the solvent viscosity is traditionally neglected (the viscosity of aqueous solvents doubles between 303 and 277 K which is the most commonly applied temperature range in the experiments). This 5 kcal/mol difference is an estimate as it only holds when Kramers theory is a good approximation to the viscosity dependence of the reaction.

As opposed to effects of solvent viscosity, little is known about how the internal viscosity is related to the energy landscape of the enzyme. Determining the temperature dependence of internal viscosity is a first step toward the integration of internal friction into the energy landscape theory. Few experiments have been published about the determination of temperature and viscosity dependence of enzyme reactions from which the temperature dependence of internal viscosity could be derived (Table 2). Although useful data were available in several studies, only a few articles draw conclusions about internal viscosity or investigate its temperature dependence.

An Arrhenius-like temperature dependence of internal viscosity of trypsin activation was found recently (14), which is in analogy to the behavior of the viscosity of liquids, which also originates from microscopic barrier-crossing events (50):

$$\sigma(T) = \sigma_0 \exp\left(\frac{\Delta E_\sigma}{k_B T}\right) \tag{3}$$

where σ_0 is a temperature-independent internal viscosity parameter, and ΔE_σ is a characteristic activation energy parameter of internal friction. The same relationship (with a different

interpretation) was found in cytochrome c folding from a collapsed state (48), and the data from intermolecular ET (9) and horseradish peroxidase dynamics (19) also support this relationship, even though they were not analyzed fully in this respect at the time of their publication.

These experimental results, which fit to Eq. (3) indicate that internal friction is related to a characteristic energy barrier. How can we reconcile this energy barrier with the energy landscape? What is its relationship to the activation energy barrier? Recently, an insightful hypothesis was concluded from folding experiments: internal friction is related to the roughness of the energy landscape (51). More specifically, based on a theoretical calculation about the effect of energy landscape roughness on the diffusional constant (52), they suggested that the measured internal viscosity difference between two spectrin domain mutants during folding was caused by the difference in the roughness of their energy landscapes (51). Their estimates about the height of the roughness, however, remain provisional, as no temperature dependence was measured.

Later, a similar idea was applied to trypsin activation and quantified based on the experimental determination of the temperature dependence of internal viscosity using Eq. (3) (14). Their model relates the flexibility of the coupling of internal and external domain movements [see Figure 2(C)] to the idea of energy landscape roughness. It suggests that the characteristic activation energy parameter of internal friction depends both on the strength of the coupling and on the structure of the landscape roughness.

An appealing feature of this model is that it attributes "meaning" to the long-known hierarchical structure of the energy landscape. However, even though it explains many experimental observations, and if it is eventually true, it is quantitative only in the energy terms. Both the quantification of domain movement coupling or flexibility, and the quantification of the structure of roughness on the energy landscape are missing. Therefore, it is only useful to explain differences between mutants of the same enzyme, which supposedly have very similar energy landscapes, and it has limited predictive power to other enzymes or reactions.

Alternative Fitting for the Viscosity Dependence of Enzyme Reactions

Even though internal friction is an appealing concept, there are cases (6,27) where it is not possible to fit the viscosity dependence data with the linear relationship [Eq. (2)], and an alternative fitting function was introduced.

The earliest observations of the viscosity dependence of the relaxation time that showed deviation from proportionality were fitted by a power function (6)

$$k(T,\eta) = \frac{A}{\eta^p}\exp\left(\frac{-\Delta E_a}{k_B T}\right), \qquad (4)$$

where p is the exponent of the power function ($0 < p < 1$). A major shortcoming of this alternative formula is that the exponent p is very difficult to interpret. This power-law dependence was explained frequently in qualitative ways, for example, suggesting that the effect of solvent viscosity is transferred by the protein or that solvent composition is modified around the protein (6,34,43). The concepts of macroviscosity and microviscosity—the viscosity of the bulk solvent and around the protein, respectively—are often mentioned, which refer to the observation that viscosity in the solution may be heterogeneous. Others gave more quantitative explanations to the power-law dependence: a frequently cited theory is the frequency-dependent friction (53). On a theoretical basis, Agmon and Hopfield (54) modeled the escape of a ligand through a fluctuating one-dimensional bottleneck where the fluctuation is influenced by the viscosity of the surrounding medium; for which Zwanzig derived a power-law dependence with $p = 1/2$ (55). Yedgar et al. established an empirical relationship between the exponent p and the molecular weight of the cosolvent (27). Even though this relationship is questionable, as they neglect different variables, as, for example, the change in water activity, their work is influential and draws attention to the fact that the properties of the cosolvent used for increasing viscosity can significantly influence the viscosity dependence of the reaction rate constant.

As the power-law dependence is a major argument for the solvent slaving model (56), a related question about the role of the solvent in protein dynamics arose recently. Frauenfelder et al. envision "essentially passive" proteins, where protein atom movements are restricted by the movement of solvent atoms, that is, the alpha fluctuations in the bulk solvent and the beta fluctuations in the hydration shell slave the rate of fluctuations within the protein. Consequently, no protein movement can occur without the solvent moving, and they deny the existence of internal friction (1). For this solvent slaving model, a picturesque analogy is introduced in the form of a traffic model in their review (57). Even though it may be an oversimplified answer [as claimed by Doster (58)] to the long existing problem [interestingly, whether the protein and the solvent should be considered as one system or separate entities was already an important question for Beece (5).] of how to integrate the effect of solvent into the energy landscape of proteins, it is an influential theory which has not been confuted convincingly by experimental evidence. However, there are indications that the picture of an "essentially passive" enzyme may not hold in general. Even though the solvent has undoubtedly important influence on protein dynamics, which is supported by many experiments and computer simulations, there is evidence for reactions taking place in glasses (6,19,40–42), in dehydrated enzyme powders (59) or in gas phase (60,61), which suggest that some movements are independent of the solvent.

Presently, we cannot decide if internal friction is a physically justified concept or alternative mechanisms [better suited to Eq. (4)] are responsible for the viscosity dependence of

enzyme reactions. An appealing feature of Eq. (2) is that it has a non-zero limit to the relaxation time of the reaction (*i.e.*, the reaction cannot be infinitely fast) at zero viscosity, as is illustrated in Figure 1. Moreover, even though the concept of internal friction is empirical at present, there are more and more ideas that link it to the energy landscape and explore its physical background. There is no agreement in the scientific community as to which relationship [Eq. (2) or (4)] is valid, or a single relationship should hold for every reaction at all. It is very challenging to distinguish the two formula in viscosity dependence experiments, as in narrow viscosity range they fit the data equally well. In wider viscosity range, the linear relationship [Eq. (2)] usually does not hold, but it is not possible to decide whether it proves the superiority of the power function [Eq. (4)] or it is due to the adverse effects of the high concentration of the cosolvents. The indecisive nature of the experimental data is recognized in works where authors analyze their data by applying several/more/different alternative fitting functions (9,10,11,17,18,20,24,48,51,56). Most probably, for a precise, physically grounded (and not only empirical) description of the viscosity dependence, a more complex relationship will have to be used, as proposed by Goldbeck in 2001: "it seems reasonable to suppose that both internal protein forces and microscopic solvent inhomogeneity can affect the coupling of bulk solvent viscosity to the coefficient of friction along the protein reaction coordinate, but the relative importance of these effects and their functional dependences on solvent and protein parameters remain open questions." (11).

Conclusion and Perspectives

Substantial amount of data has been gathered in the last 20 years since the introduction of the concept of "internal friction." It is a challenging field both experimentally and theoretically. There are two main limitations to experiments as internal friction is determined indirectly, by investigating the effect of viscosity on the rate constant of the enzyme reaction: (i) most experimental data have to be interpreted cautiously as the added cosolvents may strongly bias the results, (ii) the effect of cosolvents on parameters other than viscosity seriously limits the applicable concentration ranges, resulting in narrow viscosity ranges in the experiments. Besides these experimental limitations, there are theoretical challenges as well. In the theoretical approaches, we have to tolerate considerable simplifications due to the incomprehensible complexity of both the energy landscape and our limited knowledge about protein–solvent interactions. Depending on the type of simplification, there can be very different, often conflicting approaches (1,58). A very important milestone would be if experimental and theoretical achievements in the protein friction field were integrated into commonly accepted terms, ways of interpretations, and had practical meaning in the kinetic or dynamic study of enzymes.

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